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PATHOGENESIS OF A CASE OF CONGENITAL GOITER WITH ABNORMALLY HIGH LEVELS OF SPI AND WITH MONO- AND DIIODOTYROSINE IN THE SERUM*

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RECENT studies have demonstrated the importance of enzymatic defects in the pathogenesis of congenital goiter (1–10). Such defects may be associated with the presence of abnormal constituents in the peripheral blood. Most recently, Stanbury and coworkers (7–10) observed 3 patients with congenital goiter and low levels of serum organic iodine, in whom mono- and diiodotyrosine constituted the bulk of the circulating organic iodine. They found a defect in dehalogenation of mono- and diiodotyrosine, both within the gland and peripherally. Two of the 3 patients were siblings, and 2 of these 3 were cretinous as well as goitrous. The present report concerns a patient with congenital goiter, also with mono- and diiodotyrosine in the blood, but with a seemingly different type of defect as the cause—namely possible failure of coupling of diiodotyrosine.

The patient, a 5-year-old colored girl, had a goiter which was large and

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very vascular upon examination, a serum precipitable-iodine level of approximately 25 μg. per 100 ml., and mild hypothyroidism as estimated by body height and by the stage of epiphyseal maturation upon roentgen examination (*see* Case Report in Appendix). Partition of the serum by paper chromatography after labelling with I^{131} revealed roughly equal activities of mono- and diiodotyrosine, triiodothyronine and thyroxine,[1] as well as inorganic iodide in somewhat larger amount. These results were confirmed by chemical analysis of the several fractions from the chromatogram. Despite the finding of iodotyrosines in the serum, dehalogenase activity in the gland and peripherally was observed to be approximately normal by the techniques used by Stanbury and collaborators.[2]

METHODS

Serum precipitable-iodine (SPI) determinations were made by the original acid-ash method of Barker (11). I^{131} uptake by the thyroid was measured with a scintillation crystal directional counter placed 25 cm. from the skin over the thyroid isthmus. Serum-precipitable I^{131} (SPI^{131}) levels were determined at seventy-two hours after administration of I^{131}, using trichloracetic acid (10 per cent) as the precipitant according to a modification of the method of Chaikoff (12).

Because of the low radioactivity, a 15–20 ml. sample of serum was prepared without carrier compounds for paper chromatography and chemical determinations of the iodine content of the fractions as follows: sufficient 6N hydrochloric acid was added to the serum to bring the pH to 2.0, and the serum was poured into 4 volumes of ethanol. After standing overnight, the protein precipitate was removed and washed with 80 per cent ethanol. The protein was dried with acetone and in the air, and the radioactivity counted. The ethanol filtrate was mixed with an equal volume of chloroform and the solvent layer extracted three times with 2N ammonium hydroxide. The chloroform layer contained a negligible amount of radioactivity. The aqueous phase was concentrated in vacuo to 5 ml. and homogenized (Virtis "45") with 20 ml. of 1-butanol containing 1 drop of concentrated hydrochloric acid. The insoluble material was removed by centrifugation, washed twice with butanol, and dried with acetone and ether.

The filtrate was concentrated to dryness, then dissolved in 1-butanol, filtered, the butanol removed in vacuo, and the residue dissolved in a small volume of 0.1 N sodium hydroxide in 10 per cent 2-propanol.

The iodinated compounds were separated by paper chromatography on Whatman No. 3 paper using the ascending-descending method of Block (13), and developing (according to the procedure of Roche *et al.* (14)), first with CH_3OH: 0.2N CH_3COONH_4 (pH 6.1) = 100: 250 v/v at 0–4° C. for 50 cm. Standards of NaI, MIT, DIT, 3,5-T_2, 3,5,3′-T_3, and T_4 were also placed on the paper. After the development with methanol-

[1] Various abbreviations have been used in the literature to designate iodinated amino acids. For the sake of uniformity, we suggest the following, modified from Roche, Stanbury and others: inorganic iodine (I^-), monoiodotyrosine (MIT), diiodotyrosine (DIT), diiodothyronines (3,5-T_2; 3′,5′-T_2), triiodothyronines (3,5,3′-T_3; 3,5,5′-T_3), and tetraiodothyronine or thyroxine (T_4). Radioactivity consequent to I^{131} labelling is indicated by an asterisk (*),*e.g.*, DIT*. These abbreviations are employed subsequently in this paper.

[2] The authors are indebted to Dr. John Stanbury for his kindness in revealing this information prior to publication, and for his subsequent interest in the work.

ammonium acetate, the sheets were dried and the thyronines were separated using 2-butanol:3% NH_3=150:50 v/v (13), in the same direction as the first solvent, until the solvent front reached 2 cm. below the DIT area (24 cm.).[3] Consistently good separations were obtained by this procedure. The chromatograms were cut into strips 1 cm. wide and counted in a well-type scintillation unit.

A digest of thyroid tissue was chromatographed by the described technic after enzymatic digestion of the whole gland for four days at pH 8.4 with pancreatin (Viokase powder), 100 mg. per 150 mg. of wet thyroid tissue. The digest was concentrated to a small volume so that activity was high; it was then spotted on paper, and chromatography conducted as for serum. The left thyroid lobe used in the digest study was removed forty-eight hours after removal of the right lobe for dehalogenase studies and twenty-four hours after a test dose of 125 microcuries (μc.) of I^{131} had been administered.

Dehalogenase activity within the thyroid was tested as follows according to the technique of Roche *et al.* (15): Slices of the patient's thyroid weighing approximately 300 mg. each were incubated for five hours at 37° C. in a Warburg apparatus, using 4.5 ml. of Krebs-Ringer phosphate buffer at pH 7.3 as the incubation medium. The buffer contained DIT* in a concentration of 11 micrograms (μg.) per 4.5 ml. with a specific activity of 0.2 μc. I^{131} per 11 μg. DIT*. As control material, slices of thyroid tissue were heated for five minutes in a boiling-water bath before incubation and before adding labelled material. Following incubation, the vessels were stored for two days in a deep-freeze. After thawing, carrier I^- and DIT were added and the proteins were precipitated by the addition of 1 ml. of 15 per cent trichloracetic acid. The volume of the clear supernatant liquid was made up to 10 ml. To the supernatant solutions, 1 ml. of 1N sulfuric acid, 2 ml. of 5 per cent ferric chloride, and 4 ml. of carbon tetrachloride (CCl_4) were added. This effected the conversion of I^- to I_2. The latter was extracted from the aqueous solution with CCl by shaking for twenty minutes. The CCl_4 was removed and the dissolved I_2 was extracted from the CCl_4 with 5 ml. of 10 per cent aqueous sodium thiosulfate. The radioactivities of the 2 aqueous solutions containing DIT* and NaI^{131} respectively were measured in a well-type scintillation counter.

The patient's ability to deiodinate diiodotyrosine was also determined (9). A dose of approximately 7 μc. of DIT* in approximately 20 μg. of DIT was given intravenously.[4] Urine samples were collected at one, two, and four hours after injection, for chromatography. Blood serum, taken at four hours, was also chromatographed as a check upon the urinary findings. The excretion of diiodotyrosine was expressed as a percentage of the original dose, after correction for decay.

RESULTS

Iodinated amino acids in serum. In Table 1 are shown the relative concentrations of the iodinated amino acids identified by paper chromatography in the serum at seventy-two hours and at seven days following a test dose of 125 μc. of NaI^{131}. Also shown are the corresponding chemical analyses.

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[3] These systems have been improved by using CH_3OH:CH_3COONH_4 (pH 6.1)= 100:250 v/v at 0–4° C., and 2-butanol:conc. NH_4OH=150:50 v/v in the presence of concentrated NH_4OH vapors.

[4] This preparation contained approximately 79 per cent of its radioactivity in DIT and 21 per cent in NaI after heat sterilization.

TABLE 1. DISTRIBUTION OF LABELLED AND UNLABELLED IODINATED COMPOUNDS IN SERUM FOLLOWING ADMINISTRATION OF I^{131} AND CHROMATOGRAPHIC SEPARATION ON PAPER

Compound†	Time after injection of I^{131}					
	72 hrs.			7 days		24 hrs.‡
	%	Counts per min. at peak 1 cm. wide§	I^- peak 1 cm. wide‖	%	Counts per min. at peak 1 cm. wide§	%
Protein	9					
BUOH-insoluble	15					
T_4	11	288	5.5	8	32	
T_3	13	240	4.2	11	64	
DIT	10	320	lost	11	48	
MIT	12	256	6.0	8	32	
I^-	27	624	15.8	62	432	100¶
Total	*100*			*100*		

† For explanation of abbreviations, *see* Footnote 1 of text.

‡ Twenty-four hours after I^{131} and seventy-two hours after DIT*. This experiment was conducted nine months after those shown in the other columns. In a sample of urine studied at the same time, I^{131} was the only iodinated compound.

§ Above background.

‖ Calculated as μg. I_2 per 100 ml. of serum. Results are of comparative significance only.

¶ Although this band contained a large amount of radioactivity, no significant quantities of other compounds were observed.

Iodinated amino acids in thyroid. Table 2 and Figure 1 show the relative concentrations of the labelled iodinated amino acids within the thyroid gland five days after administration of DIT* and twenty-four hours after administration of NaI^{131}. Figure 1 also shows the results of the chemical

TABLE 2. RELATIVE AMOUNTS OF AMINO ACIDS AND IODIDE IN THE THYROID GLAND 24 HOURS AFTER ADMINISTRATION OF I^{131} AND 5 DAYS AFTER ADMINISTRATION OF DIT*

Fraction	Per Cent
T_4*	1
T_3*	Trace
DIT*	48
MIT*	20
I^{131}	31

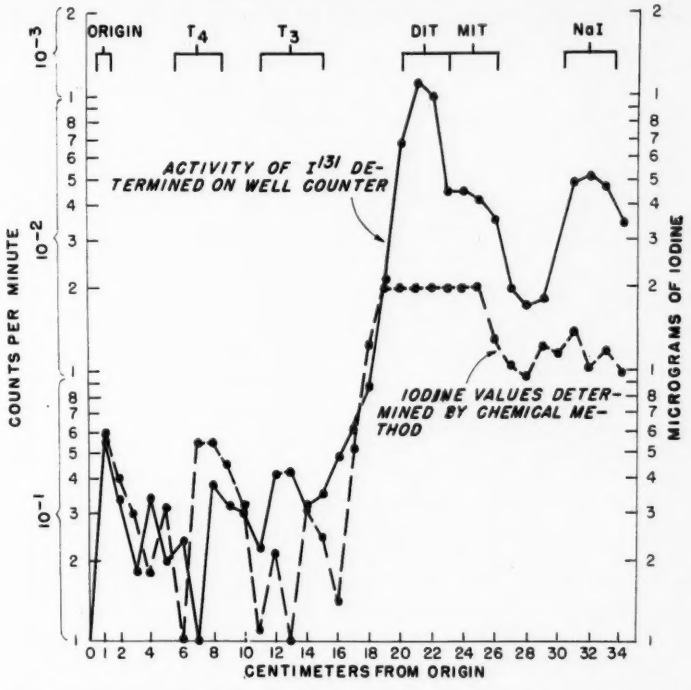

FIG. 1. Relative concentrations of labelled iodide and iodinated amino acids in the thyroid gland 24 hours after labelling with I^{131}; and of unlabelled compounds determined chemically, after chromatographic separation on paper.

determination[5] of the iodine content of these same areas on the paper. The chemical data support the results obtained with I^{131} labelling and show that no significant exchange between T_4* and the other compounds could have taken place.

Diiodotyrosine excretion. The patient excreted in the urine a total of 8.7 per cent of the DIT* (Table 3) in an unchanged form within four hours after the injection of 7 μc. This figure was somewhat higher than the upper limit of normal, 4 per cent, defined by Stanbury and associates (9). Most of the excretion occurred within the first two hours. The serum at four hours registered a weak peak of activity in the DIT* area and some traces of MIT* and I^{131}.

Dehalogenase content of thryoid. Despite a slightly increased background count resulting from the DIT* administered three days earlier, dehalogenation by thyroid tissue was demonstrable. Table 4 shows the results of

[5] The chemical method, as used, was inoperative above 200 μg. of iodine.

TABLE 3. EXCRETION OF MIT* AND DIT*† IN URINE FOLLOWING INTRAVENOUS INJECTION OF DIT* (APPROX. 2.79×10^6 C/M.)‡

	Time after injection			
	1 hr.	2 hrs.	4 hrs.	Total
Volume of urine (ml.)	15	6	84	
Count/min./ml.	1.43×10^4	2.33×10^4	4.44×10^3	
Total count/min.	2.14×10^5	1.40×10^5	3.75×10^5	
DIT* (%)§	17.2	10.9	21.2	
MIT* (%)§	10.6	16.4	14.8	
I^{131} (%)§	72.0	72.6	63.8	
DIT*+MIT*(%)§	27.8	27.3	36.0	
DIT*+MIT*:I^{131} (ratio)	1:2.6	1:2.7	1:1.8	
Total DIT*+MIT* (counts)	6.0×10^4	3.8×10^4	13.5×10^5	
Percentage of original activity as DIT*+MIT*	2.15	1.36	4.84	8.4

† For explanation of abbreviations, *see* Footnote 1 of text.

‡ The DIT, MIT, and I^- were separated on paper chromatograms. The chromatograms were scanned and the activities as indicated by the areas under the curves were used for the calculations. The results, consequently, are subject to the usual errors of automatic scanning, paper chromatography, etc.

§ Percentage of radioactivity in the urine specimen as DIT*, MIT*, etc. at 0–1, 1–2, and 2–4 hours.

TABLE 4. DEHALOGENATION OF ADDED DIT* BY THYROID GLAND TISSUE

No.	Amt. of thyroid tissue (mg.)	Tissue boiled	Radioactivity as I^{131} ($Na_2S_2O_3$ solution) (% recovered activity)	Radioactivity as DIT* (% recovered activity)
1	290	No	96	4
2	270	No	93	7
3	270	No	97	3
4	270	Yes	28	72
5	280	Yes	34	66
6	270	Yes	41	59
7	—	No	30	70
8†	280		No counts demonstrable	

† In sample 8 no DIT* was added. Although some dehalogenation had occurred during the extraction procedures (samples 4–7), it is evident from these data that the gland tissue could dehalogenate DIT to an appreciable extent.

this experiment. It may be seen that some dehalogenation occurred during the extraction procedures, but the difference when unheated tissue was employed was unquestionable.

Effects of administration of thyroid hormone. In Table 5 are listed: a) the 4-hour and 24-hour thyroidal uptakes of I^{131}, b) the SPI levels before and after the administration of *l*-sodium triiodothyronine, *l*-sodium thyroxine, and desiccated thyroid, c) the approximate size of the thyroid gland, and d) the dosage and duration of treatment. Steady decreases in gland size,

TABLE 5. EFFECT OF THYROID HORMONE ON THYROIDAL I^{131} UPTAKE, SERUM PRECIPITABLE-IODINE LEVEL, AND SIZE OF THYROID GLAND

Date	Medication			Thyroidal I^{131} uptake (% dose)	SPI (μg./100 ml.)	SPI^{131} (% orig. dose)	Approx. size of each lobe of gland (cm.)
	Preparation	Daily dose	Duration (wks.)				
June 29, '55				57 (24-hr.)	28.4	2.3 (72 hrs.)	Right 9.0×4.0×2.0 Left 9.0×4.0×2.0
July 17, '55				—	—	3.0 (48 hrs.)	
Oct. 31, '55				63 (24-hr.)	—		
Nov. 22, '55	Triiodothyronine	25.0 μg.	3	59 (24-hr.)	17.2		
Dec. 6, '55	Triiodothyronine	50.0 μg.	2	21 (4-hr.)	13.2		Right 6.0×1.5×1.0 Left 6.0×1.5×1.0
Dec. 20, '55	Thyroxine	0.20 mg.	2	13 (4-hr.)	15.0†		Right 4.0×1.5×0.5 Left 3.0×1.0×0.5
Jan. 3, '56	Desicc. thyroid	0.06 Gm.	2		10.3		No change.
Feb. 1, '56	Desicc. thyroid	0.06 Gm.	4		7.5		No change.
Apr. 28 and 30, '56	Total thyroidectomy in two stages						Each lobe about "6 cm. in diam." at operation.

† Discrepant readings in duplicate samples, with insufficient serum to repeat.

I^{131} uptake and SPI level were noted, but they did not reach the low levels seen with this treatment in euthyroid subjects and other patients with nontoxic diffuse goiter studied in this laboratory (16).

DISCUSSION

Although the patient presented as a problem in sporadic nontoxic diffuse goiter (Fig. 2) with apparent onset at the age of 3 years (*see* Case Report in Appendix) the pathologic findings and the chemical demonstration of a probable enzymatic defect in thyroxine synthesis suggest that she had a congenital goiter. Moreover the onset (during the period of study) of goiter in a distant cousin of about the same age suggests that the defect was genetically determined.

According to our results, some T_4 was synthesized and released by the patient. In fact she must have been making almost enough to maintain euthyroidism, as shown by her clinical status. This was accomplished,

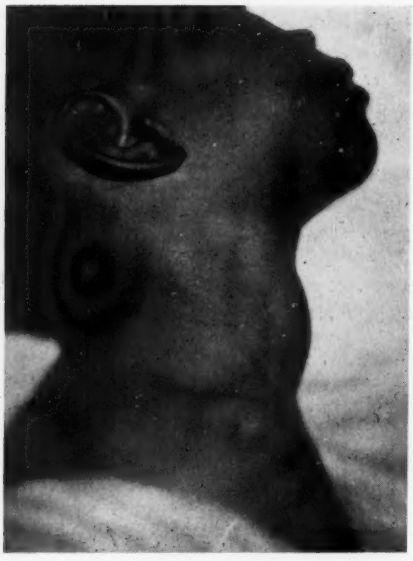

FIG. 2. Side view of the neck of Patient S.S., illustrating the fullness caused by the goiter.

however, by the development of a large goiter with greatly increased blood flow, extremely rapid turnover of iodine, and the typical pathologic findings of congenital goiter (Figs. 3–7, and Case Report in Appendix). Relatively large amounts of DIT* and MIT* were released into the blood stream, in excess of the capacity of the mechanisms for their disposal. This was indicated first, by the fact that these substances were present in the blood, and second by the unusually high levels at which they were maintained.

The presence of MIT and DIT in the circulation was not due to failure of dehalogenation. As indicated previously, tests on thyroid tissue demonstrated activity of the enzyme for dehalogenation. The enzyme was also present peripherally, as demonstrated by the limited extent of urinary excretion of DIT* following injection, although total output was slightly greater than normal. Activity of a peripheral dehalogenase was also indicated by the disappearance of all but a trace of DIT* from the blood within four hours after its administration.

The virtual absence of T_4* or T_3* from the gland and blood (Tables 1 and 2) within twenty-four hours after a test dose of I^{131} was in contrast to the significant amounts of these substances present in the blood nine months earlier within seventy-two hours after dosage with I^{131} (Table 1). This suggests a significant delay in the synthesis of thyroxine, even though the timing of the several experiments was different. The results also suggest that considerable impairment in thyroid function occurred during the nine months which elapsed between the two tests.

The best explanation that we can put forth to account for the inadequate formation of T_4 by the thyroid is a block to the coupling of DIT, once formed. As stated, the defect was not complete, since at the time of the first chromatographic separation, T_4 and T_3 were present in the serum; nine months later, the digest of the thyroid gland contained a small but definite quantity of T_4*. Oxidative coupling of DIT can occur *in vitro* without enzymatic mediation. However, most oxidative processes in the body are mediated enzymatically, so that lack of a specific enzyme is a

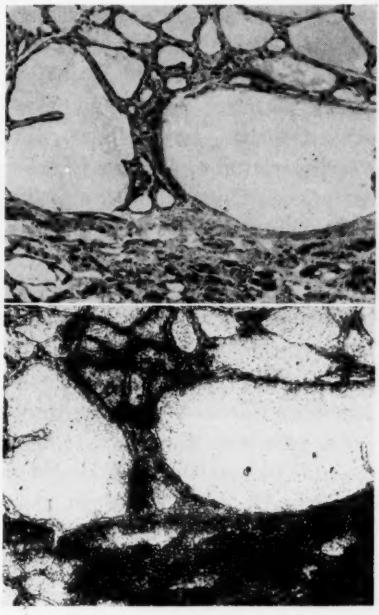

FIG. 3. Lace-like pattern of distorted, almost empty follicles with intervening small follicles. The latter show high uptake of I^{131} in the accompanying radioautograph.

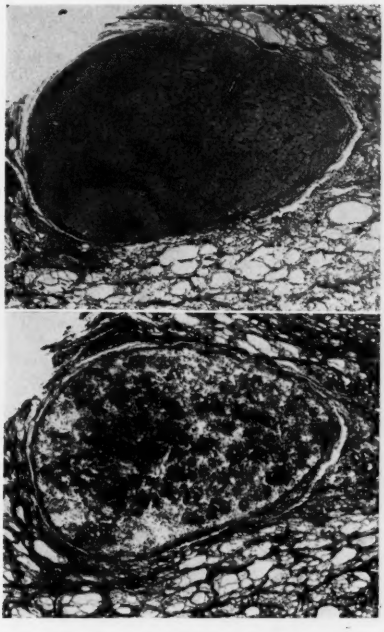

FIG. 4. Low-power view of macroscopic adenoma in left lobe of thyroid, showing high uptake of I^{131} in accompanying radioautograph.

likely explanation for a block at this step. Stanbury and Querido have made a similar suggestion in a patient with normal SPI levels (6). A less probable alternative is an inability of the gland to retain DIT. With either situation, DIT could enter the blood stream and become unavailable for coupling.

Once in the blood, DIT normally is deiodinated. I^- thus formed is re-

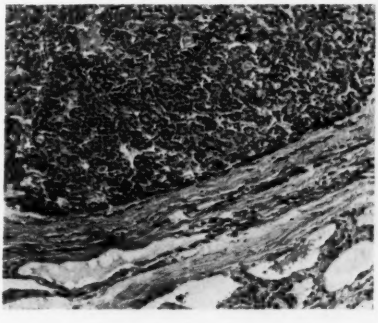

FIG. 5. High-power view of adenoma in left lobe of thyroid, showing trabecular and follicular pattern.

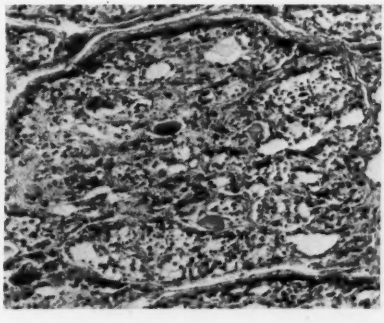

FIG. 6. High-power view of follicular and fibrous architecture in right lobe.

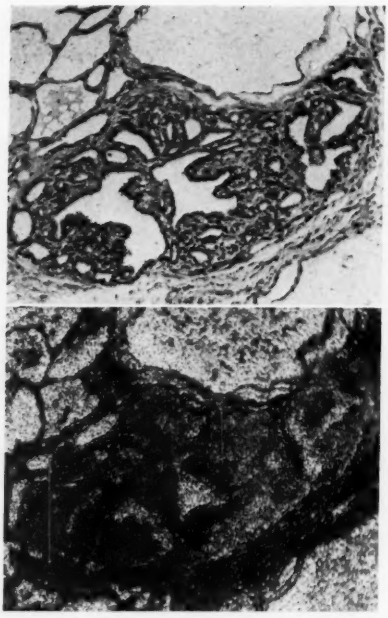

FIG. 7. High-power view of focus of papillary hyperplasia in left lobe of thyroid, showing high uptake of I^{131} in accompanying radioautograph.

turned to the gland for re-utilization or is excreted by the kidneys. In our patient, most of the iodide from deiodination must have entered the thyroid rather than the urine, despite the figures for excretion of I^{131} after DIT* administration. Thyroidal I^{131} uptake was high, on several occasions being about 80 per cent. Moreover, a rough estimate of the amount of iodine as secretion necessary to maintain the serum precipitable-iodine at the observed high level indicates that considerably more was required than could have been provided by the diet. Assuming a DIT* space of 8 liters at a blood level of 25 μg. per 100 ml., the DIT pool would have been approximately 2 mg. Assuming that 50 per cent was turned over in four hours—probably longer, as indicated by the DIT* injection experiment—the gland would have to secrete 500 μg. per hour to maintain equilibrium. The diet would probably not offer more than 150 μg. of iodide daily. Thus the bulk of the iodide must have been returned to the gland from recirculation.

The bulk of evidence suggests that derangements can occur at each step in the synthesis of thyroxine in which an enzyme is involved. At present, no correlation is possible between these enzymatic disturbances and the resultant pathologic picture. The gross and histologic changes present in our patient (*see* Case Report in Appendix) were essentially the same as those seen in other samples of congenital or juvenile nontoxic diffuse goiter of varied etiology (1–3, 5). It is clear that the thyroid can react pathologically to a prolonged stimulus with only a limited number of responses, regardless of the cause. This limited capacity for response is also in evidence in the thyroid glands of the offspring of mice given large doses of I^{131} during pregnancy (17). The pathologic changes have many points of similarity to those seen in the present patient, including the presence of nodules and adenomas.

In general, the stimulus to augmented activity of the thyroid, as well as to hyperplasia and hypertrophy, is considered to be an increased rate of

release of thyrotropin by the anterior pituitary consequent to a lowered level of circulating thyroid hormone (18). This sequence is suggested in the present patient by the fact that thyroidal I^{131} uptake, serum precipitable-iodine levels, and gland size decreased upon the administration of thyroid hormone. However, the extent and rate of suppression of these three indices was limited and slow. In most patients with nontoxic diffuse goiter and in healthy subjects, uptake and release are successfully inhibited within a week when T_3 (19, 20), T_4 (21) or desiccated thyroid (22) is given. These values become virtually zero after several weeks. This was not the case in the present patient. The data suggest that desiccated thyroid may have been somewhat more effective than either of the two thyronines.

It is likely that the delayed responses to thyroid hormone of this patient as contrasted with those in the usual patient with nontoxic diffuse goiter are simply the result of the unusual degree of pituitary secretory activity. However, it is also possible that pituitary suppression is not the only factor involved and that there may be a suppressive effect directly on the thyroid gland. Thus, the greater efficacy of desiccated thyroid might be explained by an inhibitory effect upon the thyroid gland of iodide made available from the I^-, MIT and DIT in the desiccated thyroid preparation —similar to the effect of iodides in toxic goiter.

SUMMARY

An unusual case is presented of congenital goiter with mild hypothyroidism in a 5-year-old Negress. The serum precipitable-iodine (SPI) level was approximately 25 μg. per 100 ml. Upon paper chromatography, the SPI was found to be equally distributed among monoiodotyrosine, diiodotyrosine, triiodothyronine and thyroxine. Studies of thyroidal and peripheral dehalogenation of labelled diiodotyrosine revealed the presence of dehalogenase activity in nearly normal amounts.

The best hypothesis that could be drawn to explain these findings is that there was failure of coupling of diiodotyrosine, and that this failure was caused by an enzymatic defect.

APPENDIX

Case Report

S.S. (U.H.#236124) was a 5-year-old colored girl (Fig. 2) who was first admitted to the Babies Hospital on June 29, 1955. She had been referred because of enlargement of the thyroid, known to be present for two years. At first there was no family history of goiter, but later in the study there was a report that a goiter had developed in a distant cousin of about the same age. The personal history and the record of previous health and illnesses were non-contributory. Her early growth and development had been within normal limits. The first tooth had appeared at 6 months of age. She sat alone at 5 months, walked at 14 months, was toilet trained at $1\frac{1}{2}$–2 years, and spoke sentences at 2 years.

Two years before admission, the mother first noticed that the child's neck was enlarged. The goiter continued to increase in size from then on. No systemic symptoms, local pain, or tenderness were noted. There was no history of peculiarities in the diet, or of ingestion of medicines or iodides.

In January 1955, the 24-hour thyroidal uptake of I^{131} had been 82 per cent (Dr. John Gulash of Bridgeport, Connecticut) and the serum precipitable-iodine level on two occasions, 23.4 μg. and 23.9 μg. per 100 ml.

On admission in June 1955, the patient was a normal-appearing alert girl whose height was $39\frac{1}{2}$ inches, weight $34\frac{1}{2}$ pounds, and blood pressure 100/66 mm. Hg. The height and weight were 2 standard deviations below normal. Although the head was 72 cm. in circumference, the measurement was over 2 standard deviations above normal. The thyroid was diffusely enlarged and spongy to touch, with a palpable and distinctly audible bruit over almost the entire surface. Each lobe of the gland measured approximately 9.0×4.0×2.0 cm. The teeth were erupted normally for her age. The remainder of the examination showed nothing abnormal.

Laboratory findings were: Hgb 9.5 Gm. per 100 ml; RBC 3.9 million per cu. mm.; and WBC 8,100 per cu. mm. with the percentage distribution PMN 30, L 65, M 3, E 1 and B 1. The erythrocyte sedimentation rate was 18 mm. at one hour. The urine was normal. The fasting level of serum cholesterol was 322 mg. per 100 ml., and the serum precipitable-iodine (SPI) concentration was 28.4 μg. per 100 ml. The 24-hour thyroidal I^{131} uptake was 57 per cent and the 72-hour serum precipitable-I^{131} determination revealed 2.5 per cent of the original dose of I^{131} per 100 ml. after correction for decay. (In view of the findings with paper chromatography, this suggests that at least some DIT* and MIT* were carried down by the precipitation of the proteins.) X-ray examination of wrists and hands showed a bone age of 3 years—a significant retardation in a girl of 5 years. The basal metabolic rate, determined five months later and before any treatment was given was −2 per cent by age, +6 per cent by weight and +7 per cent by weight.

A diagnosis of sporadic nontoxic diffuse goiter, presumably congenital, was made. Paper chromatographic studies were conducted to separate the iodinated compounds in the serum. The results of these determinations, as well as of later experiments designed to explore the pathogenesis of the disorder, have been reported in the body of this paper.

Following discharge on July 1, 1955, the patient was given desiccated thyroid but took the medication for one month only. Three months after discharge, in September 1955, she was 41 inches in height and weighed 38 pounds. The thyroid gland now measured about 7.5×2.0×1.0 cm. on each side.

In November 1955 she was given sodium *l*-triiodothyronine, 25 μg. daily by mouth, for three weeks. Thyroidal I^{131} uptake at the end of this time (Table 5) was 59 per cent at twenty-four hours compared with 63 per cent previously, and the SPI level at the end of treatment was 17.2 μg. per 100 ml. compared with the original level of 28.4 μg. per 100 ml. The dose of triiodothyronine was then increased to 50 μg. daily for two weeks. Each gland lobe by then was approximately 6.0×1.5×1.0 cm. in size, the SPI level was 13.2 μg. per 100 ml., and the thyroidal I^{131} uptake was 21 per cent at four hours.

In December 1955 the patient was given sodium *l*-thyroxine, 0.2 mg. daily by mouth. Two weeks later the gland measured about 4.0×1.5×0.5 cm. on the right and 3.0×1.0×0.5 cm. on the left; an audible bruit was still present on the right. The SPI level was 15 μg. per 100 ml. and the 4-hour thyroidal I^{131} uptake was 13 per cent.

In January 1956 the medication was changed to commercial desiccated thyroid, 0.06 Gm. daily by mouth. After two weeks, the gland was unchanged in size, the bruit was almost gone, and the SPI level was 10.3 μg. per 100 ml. Thyroid therapy was continued

for the next four weeks. The patient then measured $42\frac{1}{2}$ inches in height and weighed 39 pounds. The gland was approximately as before, and the level of SPI was 7.5 μg. per 100 ml. All medication was discontinued.

In March 1956, the patient was readmitted to the Babies Hospital for removal of the thyroid, but due to an intercurrent upper-respiratory infection and subsequent measles, she was discharged to her home. She was readmitted on April 26, 1956. DIT* was given intravenously for urinary excretion studies (*see* body of paper for results). The patient then underwent a total thyroidectomy in two stages, performed by Dr. Lawrence W. Sloan. The right lobe was removed three days after the injection of DIT*. Some of the tissue was used for dehalogenase studies. The left lobe was removed two days later, twenty-four hours after 125 μc I^{131} had been given by mouth. Most of the left lobe was used for chemical digestion and paper chromatography. The unused portions of both lobes were examined pathologically, and radioautographs were made. The operative and pathologic findings follow:

Operative Note. "Considering the size of the patient, this was a relatively very large, bulky gland having the general appearance of a nontoxic, diffuse goiter. The lobes were almost symmetrical and nearly spherical, except for the superior poles and a long, broad, pyramidal lobe springing from the isthmic part of the left lobe. Each lobe measured about 6 cm. in diameter. The tissue was spongy except for scattered, very firm areas, most noticeable in the lower portions of the lateral lobes. These were taken to be adenomatous structures such as had been found in the goiters of other cretins previously operated upon in this clinic. The planes were good. There was no evidence of adherence to neighboring structures or of invasion outside the capsule. No enlarged lymph nodes were noted. The gland was completely removed in two stages, on April 28th and April 30th, 1956." (Lawrence W. Sloan.)

Pathologic Findings. "Unfortunately no pathologist had the opportunity to examine the gross specimen before tissue had been removed for immediate refrigeration and paper chromatography. Two pieces of the right lobe were given in the fresh state to Dr. William Blanc of Pediatric Pathology in the Babies Hospital. These he described as follows: 'Both pieces have a similar gross appearance and consistency. They are finely lobulated, very pale tan, with a firm elastic consistency. The outer surface is homogeneous, pale tan and granular. No nodules are seen in these fragments.'

Portions of both lobes reached the laboratory of Surgical Pathology on May 2, with a request for preparation of sections for radioautographs. This was after tissue from the right lobe had been given to Dr. Blanc. The right lobe was in three parts, which were said to have weighed together 15.6 Gm. The tissue from the left lobe was in one piece and was said to have weighed 31.5 Gm. These specimens were fixed in formalin. Only one gross nodule was seen. This was in the tissue from the left lobe, and was a round homogeneous nodule 5 cm. in diameter. The lobulated regular appearance described in the fresh tissue by Dr. Blanc was evident after fixation. It is more than possible that other nodules were removed in the tissue selected for refrigeration.

Microscopically, the bulk of the tissue from the left lobe shows a lace-like pattern due to many large, distorted follicles, often empty but sometimes containing thin granular eosinophilic precipitate. These large follicles are lined by flat or low cuboidal cells, and are separated by thin trabeculae of connective tissue in which there are occasional small follicles containing colloid. The parenchyma is further subdivided into lobules by denser connective tissue trabeculae, in which groups of similar small follicles are irregularly scattered. The circumscribed nodule in the left lobe shows the pattern of a mixed trabecular and tubular type of adenoma, with considerable fibrous tissue. Some distance

away there is a small focus of papillary hyperplasia, not encapsulated. Photomicrographs and radioautographs illustrate these findings (Figs. 3–7).

The parenchyma of the right lobe is similar to that of the left. There are, however, more areas of the smaller colloid-containing follicles, and there are foci or atypical epithelium. There is one encapsulated microscopic adenoma of embryonal type in this lobe.

Comparison of these findings with those in our 7 cases of goiter in cretins shows fewer foci of adenomatous hyperplasia in this case, and more large distorted follicles. However, the variations in cells and nuclei in different fields and the lobular and nodular patterns are essentially similar.

The findings in this case are not unlike the illustrations of the histopathology in Case 5 of Wilkins and associates (4) and in the cases previously reported by Stanbury and associates (5) and presented at the meeting of the American Goiter Association on May 4, 1956 (6). It is gratifying also to learn that more than one pathologist has seen degrees of adenomatous hyperplasia in these glands with disturbed function (comparable to the adenomas frequently found in acromegalic patients) and has also been led temporarily into what is probably the erroneous diagnosis of carcinoma." (Virginia Kneeland Frantz.)

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MEASUREMENT OF THE ACTIVE IODINE STORES AND DAILY HORMONAL OUTPUT OF THE INTACT HUMAN THYROID*

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BAUMANN (1) was the first to recognize that the thyroid gland had the capacity to retain and store iodine. Other workers (2, 3) have shown a positive correlation between functional states of the thyroid gland and thyroidal iodine stores. Since these studies were performed in glands which were removed at operation or at the autopsy table, the data so derived were not applicable to the living patient except in a general way. It is apparent that an experimental method which would allow such studies *in vivo* would be extremely helpful, both in understanding the pathogenesis of thyroid disease and in helping to determine the type and amount of therapy required.

Tracer doses of radioactive iodine which are fixed in the thyroid gland can be released into the blood stream along with stable hormonal iodine in response to administered thyroid-stimulating hormone (TSH). By comparing the specific radioactivity of the newly secreted hormone with the thyroidal content of I^{131}, it is possible to estimate the hormonal stores of the intact human gland. Becker *et al.* (4) employed this technique in 3 euthyroid subjects and assumed that after five to eight days the specific activity of the circulating serum protein-bound iodine (PBI) was equal

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to that of the thyoidal compartment. In the present study the specific radioactivity of the newly secreted PBI was determined directly, so that equilibration of thyroidal and extrathyroidal PBI compartments was not necessary for valid hormonal store calculations.

METHODS

Ten healthy volunteers were each given a tracer dose of 200 microcuries of I^{131} orally. Frequent determinations of the levels of thyroidal I^{131}, serum PBI^{131} and serum PBI^{127} were made for seven to nine days. A dose of 20 units of TSH was then administered intramuscularly and the same determinations were made at intervals ranging from two to seventy-two hours. Thyroidal I^{131} and serum PBI^{131} were measured by methods reported elsewhere (5, 6, 7) and the serum PBI^{127} level was determined by the method of Brown *et al.* (8).

A sample protocol with calculations is presented in Appendix A. The *mean* specific activity of the circulating PBI for several days prior to the administration of TSH was calculated, and the specific activity of the newly secreted hormone was determined by subtracting the respective baseline PBI^{131} and PBI^{127} levels from those observed at twenty-four and/or forty-eight hours following TSH administration and comparing the rise in PBI^{131} with the rise in PBI^{127}. The thyroidal hormone stores were calculated from direct measurement of the thyroidal I^{131} content prior to TSH administration, and the calculated specific radioactivity of the newly released hormone.

The fraction of thyroidal I^{131} released daily was estimated from the serum PBI^{131} concentration after four days, and the mean thyroidal I^{131} content for this period. The details of this method will be reported elsewhere (7). The distribution space of PBI was assumed, for this purpose, to be 15 per cent of the body weight (10). The method employed to correct for PBI^{131} utilization is outlined in Appendix B. The rate of appearance of PBI^{131}, expressed as "% dose/liter/day," was converted to the percentage of the dose appearing daily in the entire body. Since the mean thyroidal content of I^{131} was determined during the same period as "% dose," the fraction of thyroidal I^{131} released per day was readily obtained. The hormonal output of I^{127} in terms of micrograms per day was then calculated as the product of the stable iodine content of the thyroid and the fractional release rate.

RESULTS

A typical study is illustrated in Figure 1, in which it may be seen that the increases in the concentrations of PBI^{131} and PBI^{127} are parallel when plotted with adjusted baseline and scale. In Table 1, the mean specific radioactivity of the circulating PBI prior to administration of TSH in the 10 subjects is compared with that of the newly secreted PBI. The circulating PBI averaged only 5.27 per cent of the administered dose per milligram of stable iodine, whereas the newly formed fraction averaged 9.87 per cent per milligram. In 7 of the 10 cases the activity in the newly secreted fraction was found to be from 1.5 to 4.3 times greater than the pre-TSH value. It cannot, therefore, be assumed that equilibrium of thyroidal and body PBI compartments had been achieved at this time.

The mean hormonal I^{127} stores are listed in the second column of Table

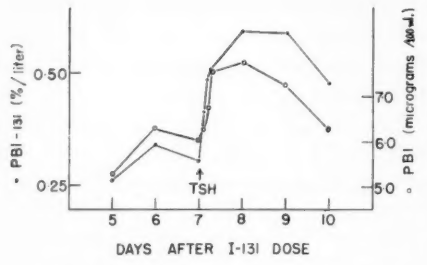

FIG. 1. Levels of PBI and PBI^{131} before and after administration of TSH in a euthyroid subject (G.M.).

2 and averaged 5.26 mg. of I^{127} per gland, with a range of 1.34 to 18.1 mg. Most of the values, however, fell below the mean of 5.26 mg. The third column lists the fractional release rate of thyroidal I^{131}, which varied from 0.0036 to 0.030 per day, with a mean of 0.0155 per day. The glands with the largest iodine stores showed the smallest fractional release rates and those with the smallest stores had the highest release rates. The product of fractional release rates and the respective hormonal I^{127} stores yields the values in the final column, which expresses hormonal output in terms of micrograms of I^{127} per day. The mean output in these 10 euthyroid subjects was found to be 56 micrograms of hormonal iodine daily, with a range of from 19 to 103 micrograms. This corresponds to a mean thyroxine output of 84 micrograms per day.

Experiments similarly performed, but in which the patients were given potassium thiocyanate and propylthiouracil prior to administration of

TABLE 1. COMPARISON OF SPECIFIC RADIOACTIVITIES OF THE BASELINE PBI AND THE NEWLY SECRETED PBI BEFORE AND AFTER THE ADMINISTRATION OF TSH IN 10 NORMAL SUBJECTS

Case No.	Specific radioactivity of serum protein-bound iodine (% dose/mg. iodine)	
	Baseline pre-TSH value	PBI released from thyroid after TSH
1	3.85	6.99
2	4.47	9.70
3	2.76	11.8
4	5.26	19.9
5	2.19	3.91
6	6.72	9.80
7	0.875	0.695
8	5.05	16.5
9	9.00	8.20
10	12.5	11.2
	Mean 5.27	9.87

TABLE 2. THYROIDAL HORMONE STORES AND SECRETORY RATES IN 10 EUTHYROID SUBJECTS

Case No.	Thyroidal I^{127} content (mg.)	Hormonal release rate (fraction/day)	Hormonal I^{127} output (μg./day)
1	2.46	0.0217	53
2	2.70	0.0109	29
3	5.09	0.00364	19
4	1.34	0.0300	40
5	3.55	0.0203	72
6	1.90	0.0246	47
7	18.10	0.00497	90
8	3.88	0.00663	26
9	3.75	0.0221	83
10	9.81	0.0105	103
	Mean 5.26	*0.0155*	*56*

TSH, yielded comparable results, indicating that the stored hormonal iodine released by TSH was formed prior to the blocking effect of these agents. In another experiment in which a subject was given a four-hour infusion of adrenaline in dosage sufficient to cause hypertension for the interval, no measurable hormonal iodine or PBI^{131} was released, although TSH was highly effective twenty-four hours later.

DISCUSSION

The statistical validity of these data was subjected to analysis and it was found that all of the elevations in PBI^{131} and PBI^{127} were in excess of 2 standard deviations from the baseline values, and most were in excess of 3 standard deviations. Since the elevations were calculated from at least 2 separate baselines and 2 post-TSH values, the validity was even greater. Whereas the magnitude of changes following 20 units of TSH was large, we were unable to obtain statistically significant differences with a 10-unit dose.

The assumption that after I^{131} administration, relatively rapid equilibration of thyroidal and serum PBI compartments occurs (4, 9, 10, 11) does not appear to be justified. In our patients, equilibration was not complete by seven to nine days. This may necessitate re-evaluation of certain studies in the literature which have made such assumptions.

The mean hormonal stores determined by this method are smaller than those determined chemically by other workers (2, 3, 12). We admit that the assumption we make of a homogeneous thyroidal iodine compartment

may well be an oversimplification, but the data of Braasch *et al.* (13) indicated that after five days the specific activity of the tyrosine fraction was greater than that of the thyroxine fraction, so that if the released hormone be thyroxine, the gland stores should be even lower than determined by this method. The discrepancy may be due to chance variation, the presence of inactive iodine stores which are not within the pathway of hormone synthesis (14), stores within the pathway of hormone synthesis but which contain little radioactivity by this time, or extrathyroidal effects of TSH. It is of interest that in one of the patients studied by Becker *et al.* (4) by a dynamic method similar to that described here, in whom a thyroidectomy was performed subsequently, the hormonal stores determined chemically and dynamically agreed within 20 per cent (15).

Although the calculated hormonal stores by the dynamic method were lower than had been suggested previously, estimates of fractional release rates were similar to those determined by other workers (10, 11, 16). Furthermore, hormone output calculations fell well within the accepted limits. Berson has estimated a hormone secretion of 68 micrograms per day (10) and 225 micrograms per day (9), whereas Ingbar found a lower value (57 micrograms per day (17)), and Stanbury calculated the output to be 70 micrograms per day (18). All of these figures are in substantial agreement and are consistent with the known therapeutic requirements of thyroxine, when one considers the potential loss of exogenously administered thyroxine. The usual dose of thyroxine required for complete replacement in myxedematous patients is in the range of 150 to 300 micrograms per day (19–22).

Patients with the lowest thyroidal iodine stores demonstrated the most rapid fractional release rates, and those with the highest stores showed the lowest rates. This is to be anticipated, since the hormonal stores depend upon the relationship of iodine collection and release rate. Since the release rate and the hormonal output are not independent variables, it is purposeless to consider their interrelationships. The baseline PBI level, however, is independent of this method of calculating the hormonal output and it is interesting that a regression line can be plotted by "the least squares method" for these variables. This line passes close to the origin and has the formula:

$$\text{PBI}^{127}\ (\text{baseline}) = \frac{\text{Hormone output}}{11.5} + 0.2,$$

meaning that for every 12 micrograms of PBI^{127} released daily one can anticipate an increase in the serum PBI^{127} level of 1.0 microgram per 100 ml. Although the number of patients studied is not large enough for significant coefficients of correlation, this relationship is of interest.

By reducing the number of determinations, this method may be modified for routine clinical use, since many observations were made in this study that were not required for the final calculations. The following minimum observations are required: thyroidal I^{131} content at one, four, and seven days after the tracer dose; level of serum PBI^{131} at four, seven, and eight days; and level of serum PBI^{127} at seven and eight days— the TSH being given on the seventh day. This method may be applied to large numbers of patients with thyroid disease, and may even be helpful diagnostically in selected problem cases.

SUMMARY AND CONCLUSIONS

A comparison of the specific radioactivity of TSH-released protein-bound iodine in the serum with that of radioactivity in the thyroid made it possible to estimate the thyroidal stores of I^{127} in 10 euthyroid subjects. These stores averaged 5.26 mg. of I^{127} per gland—a value somewhat lower than those determined by chemical methods. However, the rate of secretion of hormonal I^{127} when estimated by this method averaged 56 micrograms per day—a value in close agreement with some other estimates. Although the discrepancy in thyroid gland hormonal content between these findings and those of some other investigators may be attributed to random variation or to technical factors, it is also possible that inactive, unmeasured and physiologically unrepresentative I^{127} stores are present in the normal human thyroid.

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Appendix A

Sample data and calculations

I. *Data*

Case 1. A 19-year-old Negro volunteer. Ht., 1.75 M.; Wt., 68 Kg.

Time after I^{131} (days)	Thyroidal I^{131} (% dose)	Serum PBI^{131} (% dose/liter)	Serum PBI^{127} (μg./liter)
1	19.7	0.038	
2	—	0.045	
3	20.7	0.080	
4	—	0.133	
5	20.8	0.135	
6	—	0.190	
7	17.8	0.199	
8	—	0.235	65
9	17.2	0.256	68
TSH administered			
10	15.1	0.490	100

II. *Calculations of serum specific activity*

Serum specific activity before TSH (baseline):

$$\text{Mean serum } PBI^{127} = \frac{65+68}{2} = 66.5 \text{ micrograms/liter.}$$

Final serum $PBI^{131} = 0.256\%$ dose/liter.

$$\text{Specific activity} = \frac{0.256}{66.5} = 0.00385\% \text{ dose/microgram} = 3.85\% \text{ dose/milligram.}$$

Serum specific activity of PBI released after TSH:

Concentration of PBI^{127} released after TSH = 100.0 − 66.5 = 33.5 micrograms/liter.

Concentration of PBI^{131} released after TSH = 0.490 − 0.256 = 0.234% dose/liter.

$$\text{Specific activity of released PBI} = \frac{0.234}{33.5} = 0.00699\% \text{ dose / microgram} = 6.99\% \text{ dose/milligram.}$$

III. *Thyroidal iodine stores*:

Thyroidal I^{131} at time of TSH administration = 17.2% dose.

Specific activity of PBI released after TSH = 6.99% dose/milligram.

$$\text{Thyroidal } I^{127} \text{ content} = \frac{17.2}{6.99} = 2.46 \text{ milligrams.}$$

IV. *Fractional thyroidal I^{131} release rate*:

Body weight = 68 Kg.

PBI distribution space (estimated 15% body weight) = 68×0.15 = 10.2 liters.
Serum PBI^{131} concentration at four days = 0.133% dose/liter.
Total serum PBI^{131} in body space at four days = 0.133×10.2 = 1.36% dose.
Estimated fraction of total secreted PBI^{131} remaining after four days (*see* Appendix B) = 0.767.

$$\text{Total } PBI^{131} \text{ released by thyroid in four days} = \frac{1.36}{0.767} = 1.77\% \text{ dose.}$$

$$\text{Mean } PBI^{131} \text{ released daily by thyroid} = \frac{1.77}{3} = 0.443\% \text{ dose/day.}$$

$$\text{Mean thyroidal } I^{131} \text{ (1st 4 days)} = \frac{19.7+20.7+20.8}{3} = 20.4\% \text{ dose.}$$

$$\text{Fractional release rate} = \frac{0.443}{20.4} = 0.0217/\text{day.}$$

V. *Thyroidal hormonal iodine output*:
Thyroidal I^{127} content = 2.46 mg.
Thyroidal release rate = 0.0217/day.
Hormonal output = 2.46×0.0217 = 0.053 milligrams/day = 53 micrograms/day.

Appendix B

It was assumed that PBI^{127} production and utilization were equal and that a steady state of I^{127} metabolism existed prior to administration of TSH. Under these circumstances the release of PBI^{131} is proportional to the thyroidal I^{131} content (I_T), and the utilization of extrathyroidal PBI^{131} is proportional to the PBI^{131} remaining in this compartment (I_H). The changes in extrathyroidal PBI^{131} may therefore be expressed as:

(a) $$\frac{dI_H}{dt} = k_3 I_T - k_4 I_H$$

where t is in days,

I_T is the thyroidal I^{131} (% dose),
I_H is the total extrathyroidal protein-bound I^{131} (% dose),
k_3 is the fraction of thyroidal I^{131} released daily, and
k_4 is the fraction of extrathyroidal PBI^{131} utilized or excreted daily.

Assuming that I_T is constant--which the data showed to be essentially true during the first four days--equation (a) may be integrated to solve for I_H:

(b) $$I_H = \frac{k_3}{k_4} I_T + Ce^{-k_4 t}$$

where C, the constant of integration, is found by setting t = 0, when $I_H = 0$.

(c) $$C = -\frac{k_3}{k_4} I_T$$

and

(d) $$I_H = \frac{k_3}{k_4} I_T(1 - e^{-k_4 t}).$$

After four days the total PBI^{131} secreted by the thyroid is

$$4k_3I_T,$$

and that remaining in the extrathyroidal compartment is I_H. The fraction of that secreted which remains in the extrathyroidal compartment is (from equation (d):

(e) $$\frac{I_H}{4k_3I_T} = \frac{(1 - e^{-4k_4})}{4k_4}.$$

From our studies (7) the mean value of k_4 in normal subjects was found to be 0.139 per day, a value in agreement with that of other investigators (10). The fraction of secreted PBI^{131} in the extrathyroidal compartment four days after administration of radioiodine is, therefore;

(f) $$\frac{(1 - e^{-4 \cdot 0.139})}{4 \cdot 0.139} = 0.767.$$

The fraction utilized is the difference between this value and 1.000, or 0.233.

The serum PBI^{131} concentration at four days was multiplied by the anticipated dilution (body wt. $\times$ 0.15) and divided by the fraction of secreted hormone remaining in the extrathyroidal compartment (0.767) to obtain the total hormonal I^{131} output of the thyroid during this period. The mean daily PBI^{131} secretion (% dose) was found by dividing by 4, and the fractional thyroidal release rate was calculated by dividing by the mean thyroidal I^{131} content for this interval.

THYRO-PITUITARY RELATIONSHIPS IN CHILDREN WITH CRETINISM AND HYPOTHYROIDISM*†

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THE relationship which exists between the thyroid and pituitary has been known for a long time. Thyrotropin (TSH) production is activated in states of decreased thyroid hormone production, and anterior pituitary production of thyrotropin is inhibited by the thyroid hormone secreted. Exogenous thyroid hormone, likewise, inhibits the production of thyrotropin, resulting in decreased synthesis of endogenous thyroid hormone (1). Thus, a reciprocal relationship exists between the anterior pituitary and the thyroid gland. However, most concepts of this interaction are based on morphologic studies or, at best, on indirect physiologic evidence. There have been relatively few measurements of the levels of circulating thyrotropic hormone in humans.

In 1951, D'Angelo, Paschkis, Gordon and Cantarow (2) reported observations on the thyroid-thyrotropic hormone balance of normal and endocrinopathic individuals. Data were presented on 9 adults and 1 child with hypothyroidism. The titers of thyrotropin varied widely from patient to patient; in 2 cases they were normal, in 3 cases excessively high, and in 5 cases undetectable. Levels of thyrotropin before and after treatment with desiccated thyroid were reported for 4 of these patients. In 2 of them there was amelioration of clinical symptoms without restoration of normal hormonal balance. The present communication presents additional data on 5 hypothyroid (cretinous) and 3 euthyroid infants and children.

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MATERIALS AND METHODS

The method used for the assay of thyrotropin was that of D'Angelo and Gordon(3) Small samples of untreated serum are injected into "stasis tadpoles" and the thyroid cell-height is measured in treated and untreated controls. The degree of stimulation obtained with the unknown sample is referred to a log dose-response curve which was constructed using a purified Parke, Davis preparation of thyrotropin.[1] Sera were promptly separated and frozen at the time of collection and allowed to thaw at the time of assay. It has been established that aliquots of the same sample yield essentially similar results despite varying periods of refrigeration.

The 8 subjects were patients at St. Christopher's Hospital for Children.

PROTOCOLS

Case 1—K.M., was a 7-year-old, mentally retarded, spastic paraplegic girl. Although her bone age was estimated as 3 years, she was clinically euthyroid and her serum protein-bound iodine (PBI) level was 4.8 micrograms (μg.) per 100 ml. The hemoglobin level was 12.3 Gm. per 100 ml. The electroencephalogram and air encephalogram were normal. Earlier in life she had been treated for a two-year period with desiccated thyroid without clinical improvement.

Case 2—W.S. was a normal 3-month-old female infant who was convalescing from a mild upper respiratory-tract infection. The PBI level was 7.0 μg. per 100 ml.

Case 3—E.P. was a 9-day-old male infant who was awaiting operation for a hare lip and cleft palate, but who was otherwise normal. The PBI-concentration was 10.5 μg. per 100 ml.

Case 4—S.P. was first studied at the age of $11\frac{1}{2}$ years. He was profoundly retarded both mentally and physically. His performance level was at about 10 months and the bone age was 6 months. Roentgenographic studies of the skull showed persistence of the anterior fontanelle and numerous Wormian bones. All the deciduous teeth were present and none of the permanent ones had erupted. The hemoglobin level was 8.6 Gm. per 100 ml. The concentration of serum cholesterol was 338 mg. per 100 ml. and of alkaline phosphatase, 3.0 Bodansky units per 100 ml. Thyroidal retention of radioactive iodine at twenty-four hours was 4.6 per cent. Treatment was started with 30 mg. of thyroid daily and this was increased one month later to 60 mg. daily. During the year of treatment with thyroid, he grew $6\frac{1}{2}$ inches in height. Epiphysial maturation progressed very irregularly and was estimated to be at the 4-year level.

Case 5—E.M., a female infant, was first seen at 7 months of age because of sluggishness, persistent nasal discharge, a protruding tongue and constipation. The developmental quotient was 46 per cent and the bone age was comparable to that of a newborn. Roentgen studies of the skull showed widely patent anterior and posterior fontanelles and many Wormian bones. The level of serum cholesterol was 172 mg. per 100 ml. and of serum alkaline phosphatase, 4.8 Bodansky units per 100 ml. Retention of I^{131} by the thyroid at twenty-four hours was negligible.

Therapy was instituted with 15 mg. of thyroid daily. Owing to sporadic attendance in clinic, this was not increased to 30 mg. daily until thirteen months later, and subsequently to 60 mg. daily twelve months later. At the time of the last assay of thyrotropin there had been a good clinical response and the bone age was normal at the 10 percentile level.

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[1] One microgram of Parke, Davis preparation (50P4) is equivalent to approximately 2 milliunits of U.S.P. Reference Standard.

Case 6—R.Z. This female infant was admitted at 5 months of age because of anemia, failure to develop normally, noisy respirations and a large tongue. The developmental level was below 4 months. The level of serum cholesterol was 194 mg. per 100 ml. and of alkaline phosphatase, 0.6 Bodansky unit per 100 ml. Retention of I^{131} by the thyroid at twenty-four hours was 11 per cent. The serum protein-bound iodine level was 0.7 μg. per 100 ml. Therapy was started with 12.5 μg. of triiodothyronine twice daily. Definite clinical improvement was evident after nine days of therapy.

Case 7—K.O. was a male child in whom the diagnosis of cretinism had been established at another hospital at 4 months of age. Initially he received 8 mg. of thyroid daily which had been gradually increased to 30 mg. daily. He was maintained on this dosage until we first saw him at 22 months of age. Therapy was discontinued for a period of five weeks. Clinical symptoms of hypothyroidism recurred and the serum cholesterol concentration rose from 371 mg. per 100 ml. to 477 mg. per 100 ml. The serum protein-bound iodine concentration was 0 μg. per 100 ml. on successive occasions. Thyroid retention of I^{131} at twenty-four hours was 4.2 per cent. The developmental quotient was 46 per cent and epiphysial maturation was below the 6-month level.

Therapy was instituted with triiodothyronine, 12.5 μg. twice daily initially. Two months later this dosage was increased to 25 μg. twice daily.

Case 8—A.U. was a $7\frac{1}{2}$-year-old girl who was studied because of a goiter associated with hypothyroidism. This case is being reported in detail elsewhere (4).

RESULTS

As shown in Table 1, the levels of thyrotropic hormone (TSH) in the serum of the euthyroid 7-year-old child K.M. (Case 1) and the 2 euthyroid infants W.S. and E.P. (Cases 2 and 3) were in the same range as has been reported for euthyroid adults. The normal level of TSH in E.P. (Case 3), a 9-day-old infant, is particularly noteworthy in view of the fact that the serum PBI concentration was 10.5 μg. per 100 ml. In S.P. (Case 4), a completely athyreotic cretin who was untreated for eleven years, TSH activity was not demonstrable prior to therapy. After six months of therapy with thyroid hormone, marked thyrotropic activity was evident. After an additional period of therapy of five months' duration the serum level of TSH was still elevated, although it appeared to be returning toward euthyroid values.

Patient E.M. (Case 5), an inadequately treated 7-month-old cretin, had the highest serum level of TSH reported thus far in any patient, with the stasis tadpole method of assay. After an additional period of eleven months of inadequate thyroid therapy, the high serum level of TSH was still maintained. Definite suppression was apparent after two months of therapy with a larger dose of thyroid. Inhibition was still incomplete, however, as demonstrated by the persistently supernormal level of TSH. In R.Z. (Case 6) there was an elevated concentration of TSH prior to treatment. A short period of therapy with a relatively small dose of triiodothyronine resulted in complete disappearance of TSH from the blood. In K.O. (Case

TABLE 1. SERUM LEVELS OF THYROTROPIN (TSH) IN 5 CRETINOUS AND 3 EUTHYROID CHILDREN, AS MEASURED BY THE STASIS TADPOLE TEST

Case	Patient, sex, age, clinical status	Treatment	Mean thyroid cell-height* (μ)	Serum TSH; estim'd mean level & range (μg./ml.)
1	K.M. ♀ 7 yrs. Euthyroid	None	5.46 ± 0.09	None
2	W.S. ♂ 3 mos. Euthyroid	None	5.83 ± 0.16	0.3 (0.2–0.4)
3	E.P. ♂ 9 days Euthyroid	None	5.94 ± 0.08	0.4 (0.2–0.6)
4†	S.P. ♂ 11 yrs. Cretin	None	5.50 ± 0.10	None
		Thyroid,		
		60 mg. daily for 6 mos.	6.50 ± 0.20	1.0 (0.8–1.4)
		60 mg. daily for 11 mos.	6.23 ± 0.08	0.8 (0.5–0.9)
5	E.M. ♀ 7 mos. Cretin	None	6.92 ± 0.20	1.8 (1.3–2.0)
		Thyroid,		
		30 mg. daily for 11 mos.	7.04 ± 0.17	1.9 (1.3–2.5)
		60 mg. daily for 2 mos.	6.37 ± 0.13	0.9 (0.7–1.0)
6	R.Z. ♀ 5 mos. Cretin	None	6.11 ± 0.17	0.6 (0.5–0.8)
		Triiodothyronine, 12.5 μg. b.i.d. for 9 days	5.35 ± 0.11	None
7	K.O. ♂ 22 mos. Cretin	None	5.92 ± 0.16	0.4 (0.2–0.6)
		Triiodothyronine,	6.03 ± 0.10	0.5 (0.3–0.6)
		12.5 μg. b.i.d. for 2 mos.		
		25.0 μg. b.i.d. for 4 mos.		
8	A.U. ♀ 7½ yrs. Goitrous & hypothyroid	None	5.1 ± 0.13	None
	Normal adults		5.6 ± 0.1 (5.1 – 6.0)	0.2 (0–0.5)

* Mean and standard error.

† This case is Case 6 in Table 3 of a previous study before treatment (2).

7) the initial level of TSH was in the same range as in R.Z. (Case 6), but a larger dose of triiodothyronine administered over a longer period of time failed to modify it. A.U. (Case 8), a hypothyroid child with a goiter, was remarkable inasmuch as no TSH activity was demonstrable.

The results obtained with these sera are consistent with those previously found in hypothyroid patients. The titers of TSH varied widely from patient to patient, being either undetectable or excessively high. These data supplement those reported by D'Angelo *et al.* (2) and demonstrate the effect of therapy on the thyroid-thyrotropic hormonal balance.

DISCUSSION

The existence of a fine balance between the pituitary and the thyroid has been repeatedly suggested by many investigators. Much indirect evidence has been accumulated to support this concept. The findings in this clinical study, like those of other studies (1), do not conform to the results expected if a reciprocal regulatory control between thyroid and hypophysis were operative in the strict sense.

The absence of TSH activity in patients with hypothyroidism has been reported before. Starr, Rawson and their colleagues (5) in 1939 reported the absence of TSH activity in the urine of a 55-year-old woman with longstanding myxedema. During early treatment it appeared in large quantity and disappeared after treatment had maintained the patient in a euthyroid state. In 1940, Salter (6) reported the absence of TSH activity in the urine of a 40-year-old woman with obvious myxedema prior to therapy, but TSH became "abundant" after therapy. In 1951, D'Angelo, Paschkis and colleagues (2) reported that there was no TSH activity in the serum of 5 out of 10 hypothyroid subjects. Since post-treatment values were not obtained in all of these patients and since the thyroid response to exogenous TSH was not studied, one cannot be certain that at least some of these patients did not have a primary pituitary deficiency of thyrotropic hormone. Gilliland and Strudwick (7) have recently reported a similar observation. The TSH assay was performed by measuring the discharge of I^{131} from the thyroid of day-old chicks (8). Although in normal persons the serum concentration of TSH is not always measurable by this method, elevated levels of TSH were demonstrable in 8 out of 10 adults with spontaneous myxedema. Two of the patients had no demonstrable TSH in the serum. One of these was a 63-year-old woman in whom the myxedema was controlled with replacement therapy until seven weeks before her second admission because of a relapse after omission of thyroid therapy; at this time there was a high level of serum TSH. The second patient was not studied after treatment. Two cretins, aged 12 and 17 years, were studied and found to have very high levels of serum TSH activity; in 1 of these, the level was not measurable after therapy. Two patients with

surgically induced myxedema of three months' duration also had high levels of TSH in the serum. S.P.(Case 4) of this study also demonstrates very clearly the complete lack of TSH in a severe case of cretinism, untreated for a long period of time. After therapy with thyroid hormone, very high levels of TSH were demonstrable in the serum.

This observation is not completely surprising. The absence of pituitary acidophils—the presumed site of growth hormone synthesis and secretion —in some species of thyroidectomized animals has been long known. Replacement therapy with thyroxine results in maintenance of the acidophils, secretion of growth hormone and an increase of body weight. Eartly and Leblond (9) have also presented evidence that in the hypophysis of the thyroidectomized rat there is a decreased secretion, not only of growth hormone but of gonadotropic and corticotropic hormones. Many observations indicate that the secretion of thyrotropin is increased in states of decreased circulating thyroid hormone (1). It is not known whether this continues indefinitely. Moreover, it is conceivable that the pituitary and/or hypothalamic centers eventually participate in the generalized depression of metabolic activity resulting from absence of the thyroid hormone. With replacement therapy, the pituitary and/or hypothalamus may share in the general bodily improvement, with heightened metabolic activity and renewed ability to synthesize and release thyrotropin. Once pituitary and/or hypothalamic activity is re-established, there may be a time-lag before the pituitary-thyroid interaction becomes adjusted in its homeostatic mechanism. Thus, elevated serum levels of TSH may persist for variable periods of time after circulating thyroid hormone levels have returned to normal

Gilliland and Strudwick (7) state that the occurrence of high serum concentrations of TSH in their 2 cretins may have been related to the youth of these patients (aged 12 and 17 years). However, Patient S.P. (Case 4) in the present study was only 11 years of age, and had no demonstrable TSH before therapy; he was, however, profoundly hypothyroid. This suggests that not only the duration of the hypothyroidism but also its severity may be a factor in determining the interval before "myxedema of the pituitary" results in a deficiency of thyrotropin. More data will be required before the temporal relationships of this disturbance in pituitary-thyroid interaction can be clearly delineated.

Failure to demonstrate an elevated serum level of TSH in a 9-day-old infant with a PBI level of 10.5 μg. per 100 ml does not support the suggestion that the elevated serum protein-bound iodine levels consistently found in newborn infants may be secondary to increased secretion of TSH (10). Phillips and Gordon (11) studied the functional relationship between the thyroid and the pituitary in rats which were 1 to 15 days old. They emphasize the importance of assaying the sera for TSH before attempting

any interpretation of the functional state of the pituitary gland. They demonstrated that the pituitary and thyroid glands of immature rats are very labile and respond with sensitivity to environmental stimuli and metabolic demands. This may be true of the immature human subject also but more observations are needed before any definite conclusions are justifiable.

SUMMARY

Serum levels of thyrotropin in a group of euthyroid and hypothyroid infants and children before and after therapy with thyroid hormone are reported. Absence of thyrotropic activity in severe hypothyroidism and its presence after thyroid therapy have been demonstrated and the significance of this observation is discussed. Before attempting any final interpretation of the functional state of the pituitary in hypothyroid states, it is important to measure the level of thyrotropic activity in the serum.

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DAILY FLUCTUATION OF THE SERUM PROTEIN-BOUND IODINE LEVEL

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DETERMINATIONS of the concentration of protein-bound iodine (PBI) in serum are being widely used as a measure of thyroid activity. Although the normal range is generally accepted as 4–8 micrograms per 100 ml. of serum, no information is available as to the normal fluctuation of the PBI is one individual from day to day. If great reliance is to be placed on a single PBI result, then it is of importance that the circulating level of the PBI remain fairly constant. Danowski *et al.* (1) studied the PBI in 4 females and 2 males at about weekly intervals for one and a half to two months. Variations in the females ranged from 0.5 to 0.8 microgram per 100 ml. and in the males from 0.3 to 1.1 microgram per 100 ml. The authors concluded that the PBI level appeared to be relatively constant over a period of weeks in healthy young adults.

MATERIALS AND METHODS

The subjects were 2 males and 3 females. All the females were in the reproductive period, had normal regular menses, and were studied for slightly more than one complete menstrual cycle.

Each day at about the same time blood was drawn from each subject for approximately thirty consecutive days. In addition, in the case of 1 female, additional blood was drawn for hematocrit determinations in order to determine whether a hemoconcentration or hemodilution had occurred. In the cases of 2 of the females, basal body temperatures were measured throughout the cycle in order to determine the time of ovulation (indicated on the graphs by the beginning of the luteal phase).

PBI determinations were performed in duplicate by a modification of an alkaline-ash incineration procedure (2). As routine controls on the method, pooled serum and internal standards were run with each batch constituting a separate colorimetric group. Inasmuch as the interpretation of results of such a series is wholly dependent upon the accuracy and reproducibility obtained, statistical analyses were made.

RESULTS

The results of the five series are shown in Figures 1 and 2. It may be noted that the serum PBI level was not constant from day to day in any

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of the subjects studied. The data were analyzed statistically by analysis of variance. The determinations for PBI were performed in duplicate, and for all subjects except No. 3 the daily serum specimens were stored at refrigerator temperature and analyzed in batches of approximately 14 successive samples. The stability of such samples had been previously established at refrigerator, or even room temperature. The results for each

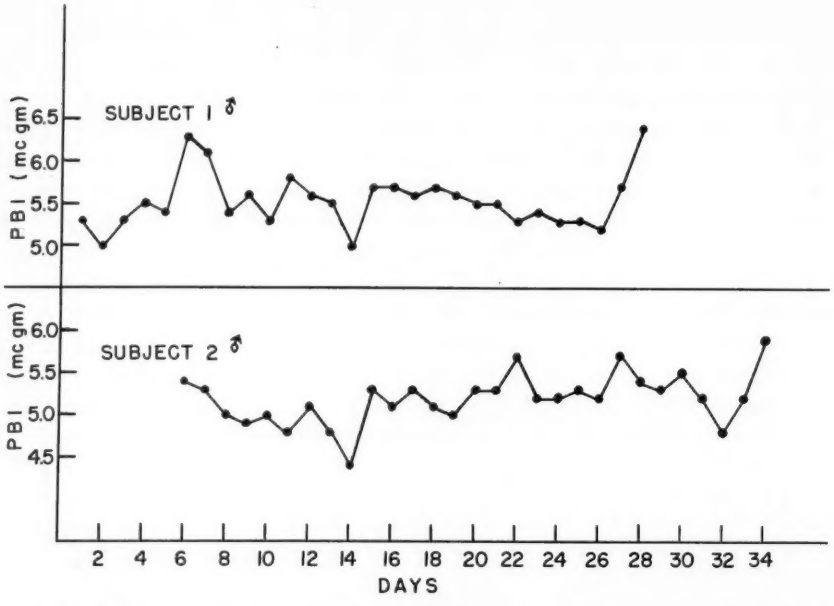

FIG. 1. Daily serum PBI values obtained for 2 male subjects. The values for Subject No. 2 have been shifted to the right to show the possible similarity in pattern with those for Subject No. 1.

batch were subjected to analysis of variance and the components of variance (between days and error variance) for each batch then pooled for estimates for each subject. The purpose of this approach was to eliminate the "between-day" variance in the analytical method itself. The samples in the case of Subject No. 3 were run in duplicate on successive days, rather than in batches, so that the estimate of error derived from the duplicate results would not be necessarily valid. Accordingly, the "between-day variance" for this subject was compared with an independent estimate of "between-day variance" derived from results of pooled serum standards run daily in the laboratory routine. The results of the analysis of variance are shown in Table 1. It is seen that the probability that the

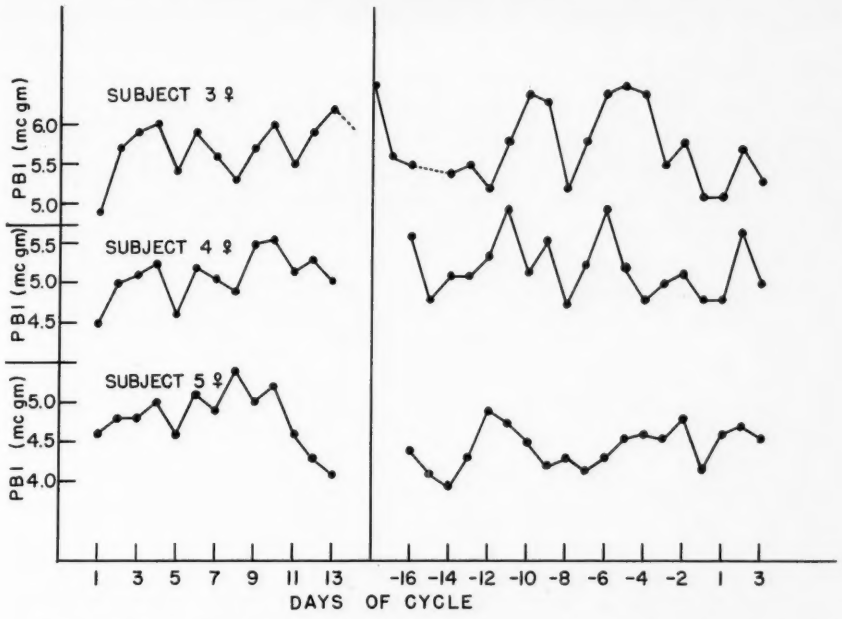

FIG. 2. Daily serum PBI values obtained for 3 female subjects. To show the similarity in pattern, the follicular phase is plotted from the first day of menses, whereas the luteal phase is plotted back from the first day of the next menses. This is necessary because of the different length of cycles. Note particularly that in the first seven days for the 3 subjects the curves follow the same pattern, and that in the first 11 days for Subjects Nos. 1 and 2 the curves are similar.

differences between days are due to analytical error is less than 1 chance in 20 for Subject No. 2 and less than 1 chance in 100 for the other 4 subjects. The 95 per cent confidence limits (2 coefficients of variation) for the average of duplicate determinations also are listed in the last column of the table.

TABLE 1. ANALYSIS OF VARIANCE

Subject	Mean square		F ratio	Probability of significance	2 CV of $\bar{x}$ of duplicates
	Between days	Error			
1 ♂	0.234	0.036	6.5	$P < 0.01$	4.9
2 ♂	0.134	0.057	2.3	$P < 0.05$	6.6
3 ♀	0.416	0.062	6.7	$P < 0.01$	
4 ♀	0.177	0.031	5.7	$P < 0.01$	4.9
5 ♀	0.164	0.039	4.2	$P < 0.01$	6.0

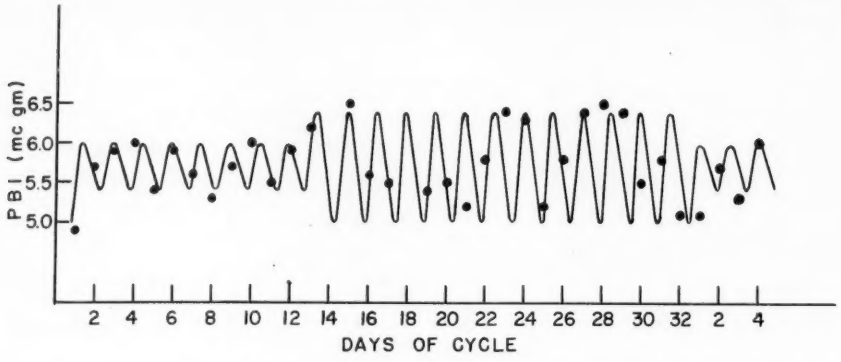

FIG. 3. Fourier analysis of the data for Subject No. 3 shows a periodicity of approximately 1.5 days. Note that the amplitude of the range of PBI values in the luteal phase is greater than in the follicular phase.

It would appear from inspection of the 5 series that the fluctuation is not random, but suggests an orderly process. In order to verify this impression, the data of the females were submitted to a Fourier analysis, the results of one typical analysis being shown in Figure 3. The following facts are apparent from these data: 1) The curve shows a major frequency of approximately 1.5 days, and 2) the amplitude of the curve in each of the female subjects is higher during the luteal phase than in either the preceding or following follicular phases. Danowski *et al.* (1) also cautiously suggested a trend toward a mid-point rise followed by a decrease during the postovulatory phase, based on their data regarding PBI levels determined at weekly intervals.

It is probably significant that the PBI curves for the 2 males are similar (Fig. 1), that the curves for the 3 females resemble each other (Fig. 2), and that there is no similarity between the curves for males and those for females.

The order of magnitude of the difference between the maximum and minimum value obtained for each subject (1.5 micrograms) is of interest and is remarkably consistent, although data from additional subjects would undoubtedly vary around this value.

There was no significant variation in the hematocrit determinations performed on Subject No. 4, demonstrating that the variations in the PBI level, in this individual at least, were not dependent upon hemoconcentration or hemodilution.

CONCLUSIONS

The data indicate that there is a significant day-to-day variation in the serum PBI level and, from the results on these 5 subjects, it would appear

that this variation is cyclic rather than random. Furthermore, this periodicity is no simple function.

From these data it is evident that a single determination of the PBI level is not definitive, and there must be some latitude in its interpretation, since each individual has a range within which a single determination will fall. This also suggests the possible necessity for re-evaluation of the definition of the range of PBI concentration in euthyroid subjects.

This evidence does not invalidate the usefulness of the PBI determination, but broadens our concept of thyroid physiology as a dynamic mechanism. The significance of possible deviations from the normal oscillation remains to be studied.

SUMMARY

Determinations of the level of serum protein-bound iodine were performed on consecutive days for a period of at least one month in 2 males and 3 females. The data indicate that there is a significant day-to-day variation in the PBI level, which appears to be cyclic rather than random.

In the female subjects during the luteal phase of the cycle, the PBI level was higher and the oscillation in values was greater.

Acknowledgments

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MARKED DEPRESSION OF THE PLASMA PROTEIN-BOUND IODINE CONCENTRATION IN THE ABSENCE OF CLINICAL HYPOTHYROIDISM DURING TESTOSTERONE MEDICATION

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THE administration of steroids, including estrogens, cortisone and prednisone, has been shown to be associated with changes in the level of circulating protein-bound iodine (PBI), thyroidal iodine uptake, and plasma thyroxine-binding capacity (1–4). The present article reports marked depression in the level of circulating plasma PBI, unaccompanied by clinical evidence of altered thyroid function during the administration of testosterone.

CLINICAL MATERIAL AND METHODS

Patients

Six patients (#1–6) were dwarfed prepubertal children. The cause of dwarfism was not determined, but it was associated with gonadal dysgenesis[1] in Patient #1, a 4-year-old female. A nutritional and/or genetic component may have been present in Patient #2, a 6-year-old male.[2] A genetic component was probably present in Patient #3, a 6-year-old male; he also had multiple café-au-lait spots but no other signs suggesting neurofibromatosis. Patient #4, a 7-year-old male, had premature synostosis of the sagittal suture and moderately severe dolichocephaly associated with hearing loss. In Patient #5, an 11-year-old female, there may have been a mild nutritional component in the dwarfism; evidence of puberty appeared shortly after testosterone was started. In Patient #6, a 7-year-old female with female sex chromatin, there were no abnormal findings other than mild dwarfism.

Seven patients (#7–13) were female adults. They had advanced cancer of the breast, but were not critically ill during the period of testosterone medication.

Methods

The prepubertal children (#1–6) received 25 milligrams of methyltestosterone orally three or four times daily for about forty days. The women with mammary cancer

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[1] Typical clinical findings, and the demonstration of male sex chromatin pattern in the cells of the buccal epithelium.

[2] When the "weight age" was less than the "height age," a nutritional component in the dwarfism was considered. When one or both parents were obviously short (father less than 66 inches, mother less than 60 inches), a genetic component was considered.

(#7–13) received 50 milligrams of testosterone propionate intramuscularly three times a week for periods ranging from fifteen to fifty-five days. Diet was not controlled.

The method of Barker (5) was used for determination of the plasma concentration of PBI in Patients #1–6, and the method Zak as modified by Leffler (6–7) was used in Patients #7–13. All determinations were performed in duplicate and recoveries were included with each set of analyses. The analytical variability of the results in duplicate samples was usually found to be within 0.4 gamma per 100 ml. of plasma. The normal range of plasma PBI values for adults was taken from the data of Starr *et al.* (8). The lower limit for the concentration of PBI in normal children is thought to be higher by 0.5 gamma per 100 ml. than that in adults (9).

The 24-hour thyroidal I^{131} uptake was corrected for extrathyroidal activity by subtracting activity measured over the thigh. The I^{131} uptake in normal subjects is from 15 to 50 per cent.

RESULTS

Prepubertal children receiving methyltestosterone orally (Patients #1–6)

The control plasma PBI concentrations in the 6 prepubertal children were within the normal range. During oral methyltestosterone medication, there was a drop in the PBI level in 5 of the 6 cases—in 4 instances to with-

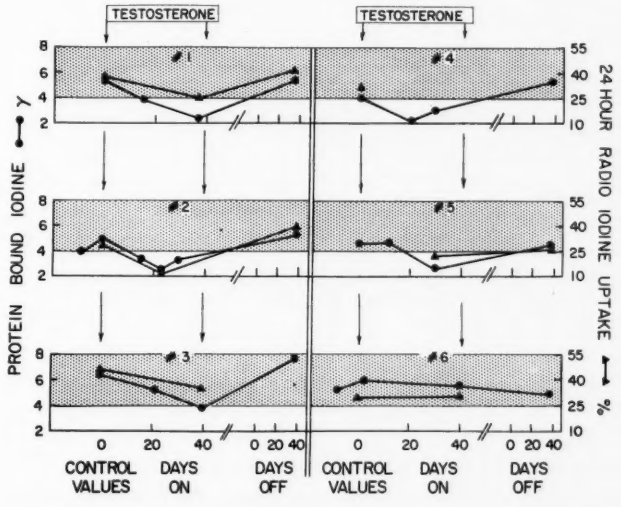

FIG. 1. Plasma PBI concentration (γ/100 ml.), and 24-hour thyroidal I^{131} uptake (% dose) in 6 prepubertal children during the oral administration of methyltestosterone. The shaded area covers the range of PBI values in normal children. In Patients #1–5 there was a decrease in the concentration of PBI, reaching hypothyroid levels as early as the twelfth day. The thyroidal uptake of I^{131} fell in 4 of the 5 cases in which it was determined with and without therapy, but did not decrease definitely below the normal range except in Patient #2. In this child an uptake of 14 per cent was observed on the twenty-second day of therapy.

in the hypothyroid range (Fig. 1). The fall in PBI concentration occurred as early as the twelfth day and was sustained during the next three weeks. After methyltestosterone was discontinued, the PBI level returned to the normal range by the thirtieth day.

The 24-hour thyroidal I^{131} uptake was determined with and without testosterone in 5 of the 6 children (Fig. 1). In the 4 of the 5 (Nos. 1, 2, 3 and 5), a lower uptake was observed during administration of testosterone, but in only Patients #2 did the uptake fall definitely below the normal range. In the fifth patient (No. 6) there was no change.

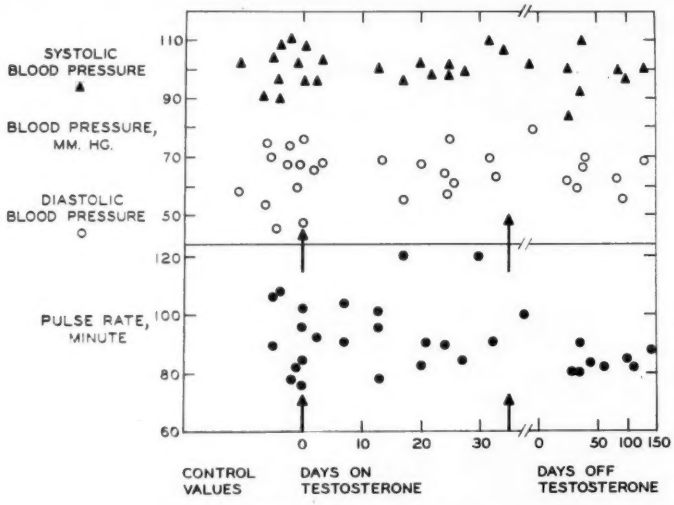

FIG. 2. Blood pressure readings and pulse rates in 6 prepubertal children, before, during and following administration of methyltestosterone.

In the group as a whole, weight gains of 10 to 15 per cent and an increase in appetite and activity were noted. No untoward reactions occurred, except the development of a moderately pronounced acneform rash and a deepening of the voice in Patient #2. The pre-breakfast blood pressure readings and pulse rates underwent no change during testosterone medication (Fig. 2). Although the skin tended to become pinker, there was no change in texture. No tendency toward constipation was noted.

Women with mammary cancer receiving testosterone propionate intramuscularly (Patients #7–13)

The findings in these 7 women (Fig. 3) were similar to those in the 6 prepubertal children. In all 7, there was a drop in the level of plasma PBI

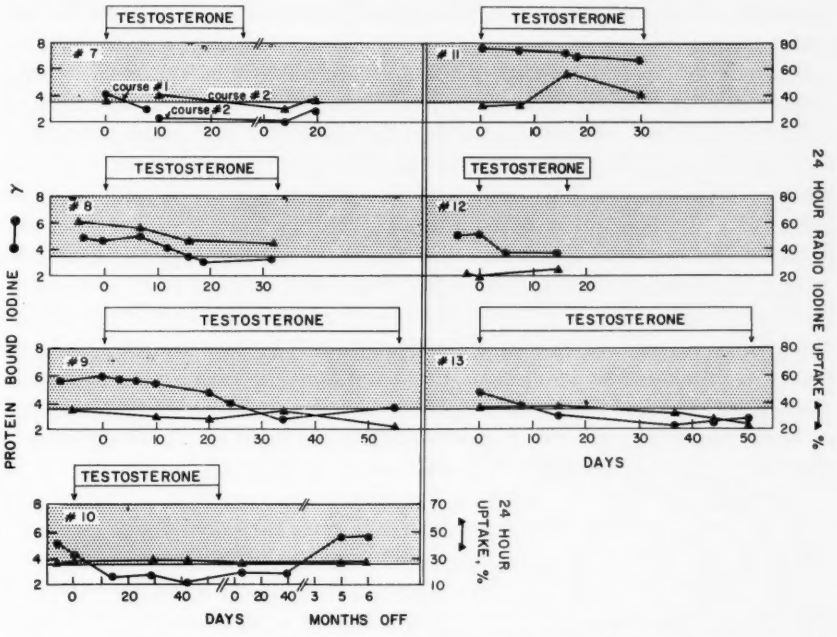

FIG. 3. Plasma PBI concentration (γ/100 ml.) and 24-hour thyroidal I^{131} uptake (% dose) in 7 adult women with breast carcinoma, during administration of testosterone propionate intramuscularly. The shaded area covers the range of PBI values for normal adults. There was a decrease of the PBI level in all 7 patients—to within the hypothyroid range in Patients #7, 8, 9, 10 and 13. The thyroidal I^{131} uptake fell in 4 patients, was unchanged in 2 and rose in 1 (#11).

during testosterone therapy; in Patients #7, 12 and 13 it occurred by the eighth day. In 5 of the 7 women (#7, 8, 9, 10 and 13) PBI values in the hypothyroid range were observed during the administration of testosterone. In the patient (#11) who had the highest initial PBI level, there was a fall which just exceeded the methodologic variability.

The thyroidal I^{131} uptake in Patients #7, 8, 9, and 13 fell slightly during testosterone medication, but in Patients #10 and #12 there was no change. In the only patient (#11) who was studied for more than three weeks and who did not have a pronounced fall in PBI concentration, there was a rise in thyroidal I^{131} uptake during testosterone therapy.

In this group there were no clinical changes of significance.

DISCUSSION

Hypothyroidism usually is associated with a reduction in the level of circulating PBI, a reduced BMR, decreased thyroidal iodine uptake, and a

characteristic clinical picture. The decrease in the plasma PBI level in the present patients was not accompanied by clinical features of hypothyroidism. In Patients #1–6, the serum alkaline phosphatase level, which in children is reduced during hypothyroidism (10), remained unchanged or rose (11). In 2 of the 7 adult patients the absence of hypothyroidism was also indicated; during testosterone medication the BMR rose in Patient #9 and remained unchanged in Patient #12. In other series the BMR has been reported to remain unchanged or to rise from 10 to 20 points during administration of testosterone (12–15). A fall in the circulating PBI level during testosterone medication might occur as a result of: 1) a change in the thyroxine binding capacity of plasma, 2) a change in renal iodine clearance, 3) increased utilization of thyroxine, or 4) decreased thyroidal output of thyroxine. In the absence of clinical and laboratory signs of hypothyroidism, the low PBI concentration can best be explained by the first of these mechanisms. A decrease in thyroxine binding by serum proteins has been suggested from the data on 2 patients receiving testosterone (16).

A low level of circulating PBI in the absence of hypothyroidism has also been found in patients with hypoproteinemia (17, 18). However, it is not likely that hyproteinemia develops in patients receiving testosterone, since this steroid is a protein anabolic agent, and the protein concentration of plasma has been reported to undergo little change during testosterone administration (19–20).

The decrease in thyroidal radioiodine uptake could have resulted from an increased renal clearance of iodine (21, 22). This possibility is strengthened by the finding that the urea clearance increased (on the average, 30 per cent) during methyltestosterone therapy in Patients #1–6 (11). It could also have resulted from suppression of the thyroid-stimulating hormone, as has been suggested by Gassner (23).

The finding of decreased PBI concentration, decreased thyroidal I^{131} uptake, and absence of clinical hypothyroidism can be explained if administration of testosterone results in an increase in the level of circulating "free" thyroxine and if the rate of secretion of the thyroid-stimulating hormone by the pituitary is dependent on the circulating level of "free" thyroxine. Another possibility is that the anabolic effect of testosterone masks mild clinical hypothyroidism. The elevated plasma cholesterol concentration seen in some patients receiving testosterone can be cited to support the case for hypothyroidism (11, 24). Moreover, it should be borne in mind that clinical symptoms of hypothyroidism are slow in development. It may not be possible to establish the true thyroid status during testosterone administration until the circulating "free" thyroxine concentration is known.

The data of Brown-Grant (25) indicate that testosterone does not change

the kinetics of I^{131} transfer in the thyroid gland, but Money and associates (26) have shown that the intramuscular administration of 5 milligrams of testosterone a day for ten days to a 300-gram rat results in an increase in the thyroidal I^{131} uptake. However, considering differences in the size and duration of dosage, it cannot be concluded that there is a species difference in the response to testosterone. Zingg and Perry (27) failed to observe an effect of testosterone of the thyroidal I^{131} uptake in humans, but their studies were carried out after testosterone had been administered for only six days.

Evidence has been presented that a rise in PBI concentration occurs during pregnancy and during the administration of large amounts of estrogens (1, 28–32). The absence of clinical hyperthyroidism has been explained on the basis of an increase in thyroxine binding by the serum proteins (33, 34). On the other hand, it has been shown that corticoids increase the blood cholesterol level and depress the PBI level and thyroidal I^{131} uptake (1–4, 35–38). The term "corticogenic hypothyroidism" has been used to describe the laboratory findings, but the patients usually do not have a clinical picture suggestive of hypothyroidism (35, 36).

It will be of interest to see whether there is also a decrease in the PBI level during the administration of large amounts of testosterone to normal adult males. The finding of Man *et al.* suggests (38) that the concentration of the circulating PBI is the same in adult males and females.

SUMMARY

In 12 of 13 women and children given either testosterone propionate intramuscularly or methyltestosterone orally for periods usually ranging between six and nine weeks, there was a reduction in the level of plasma protein-bound iodine—in 9 instances to within the range observed in hypothyroidism.

In 8 of the 10 instances in which determinations were made before and after therapy, the 24-hour thyroidal I^{131} uptake decreased following the administration of testosterone.

In no patient was there clinical evidence of hypothyroidism. The possible mechanisms accounting for the low concentration of circulating PBI in the absence of clinical signs of hypothyroidism are discussed.

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CYCLIC VARIATIONS IN THE EOSINOPHIL COUNT DURING THE PHASES OF THE MENSTRUAL CYCLE

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INTRODUCTION

MENSTRUATION is initiated and maintained by a complex coordinated activity of the endocrines. Its various phases are known to be associated with many hematologic changes, both biochemical and cytologic (1). The total leukocyte count of peripheral blood increases during the menstrual flow (2). Opinion varies regarding the behavior of the level of circulating eosinophils, and both eosinophilia and eosinopenia have been reported during the menstrual period.

The relationship of the adrenal cortex to some reproductive functions is well known (3). Recently, widespread changes in serum electrolyte levels have been reported during the menstrual cycle, presumably due to alterations in adrenocortical activity (4). The menstrual cycle includes two significant events—ovulation and vascular crisis—which may work as endogenous stressing agents. The present work was undertaken in order to obtain comprehensive data regarding the level of circulating eosinophils during the various phases of the menstrual cycle and to find out whether the circulating eosinophil level could provide any indication of the pattern and degree of variability of adrenocortical activity.

MATERIAL AND METHODS

Ten healthy unmarried female students aged 17 to 22 years, with a history of normal regular menstruation, were studied over a period covering two consecutive menstrual cycles.

The eosinophil leukocytes were counted by Pilot's phloxine technique (5) as adapted in this laboratory (6). The time of 12:00 noon was selected for conducting all counts, as this is the time when the level of circulating eosinophils is least disturbed due to diurnal variations (7).

The eosinophil counts were conducted on ten of the days in each menstrual cycle. These ten days were arranged to cover the whole cycle, so that the counts were obtained for one day each during the early and the late phases of the menstrual period and for two days each both during the early and late parts of the follicular and luteal phases, respectively, of the intermenstrual period.

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The interval between the cessation of bleeding and the fourteenth day from the day of onset of menstruation was taken to represent the follicular phase, whereas the interval between the fourteenth day and the day of onset of the next menstruation was taken to represent the luteal phase. No definite attempt was made to differentiate between ovulatory and anovulatory cycles.

RESULTS

The values for the eosinophil cell counts obtained during the different phases of the two consecutive menstrual cycles in each of the 10 subjects are presented in Table 1.

The eosinophil counts were highest during the late part of the menstrual phase in 18 of the 20 cycles studied. In the remaining 2 cycles the highest values were observed in the early part of the follicular phase. The lowest values were obtained during the luteal phase in 14 cycles, and during the early part of the menstrual phase in 6 cycles. During the follicular phase the eosinophil counts were intermediate in value. Thus the level of circulating eosinophil cells is subject to regular fluctuations during the different phases of a menstrual cycle. It was also observed that there was a progressive fall in the level of eosinophils during the follicular and luteal phases (Fig. 1).

To calculate the degree of change in the eosinophil level it is necessary to know the average values during the different phases. In Table 1 the two readings listed in the columns labelled early and late parts of the follicular and luteal phases were obtained on two different days. An average of these two readings can be taken to represent the average number of eosinophils during that part of the particular phase of the cycle. Thus the 8 counts made during the intermenstrual period of each cycle were reduced to 4 values representing the average number of eosinophils during the early and late parts of the follicular and luteal phases. These 4 average values for the eosinophil counts in the intermenstrual period plus the 2 values for the counts obtained for the early and late parts of the menstrual phase furnish 6 values which represent the level of eosinophil cells during the early and late parts of the three principal phases of the menstrual cycle. To obtain average representative data for a particular subject, these 6 values of the first cycle were again averaged with the corresponding 6 values obtained for the second cycle of the same subject. Thus the 20 counts conducted for each person during the two cycles were reduced to 6 values which represent an average of the average values of eosinophil counts for the corresponding phases of the two cycles. The over-all average values were calculated for each of the 10 subjects, and are shown in Table 2. It is significant that this procedure did not alter the character of the change in the eosinophil level as brought out by the original data in Table 1. The percentile fall in the level of eosinophils during the different phases of a cycle was calculated

TABLE 1. EOSINOPHIL COUNTS PER CUBIC MILLIMETER OF BLOOD DURING THE VARIOUS PHASES OF TWO CONSECUTIVE MENSTRUAL CYCLES OF 10 SUBJECTS

Subject No.	Cycle	Menstrual phase		Follicular phase				Luteal phase			
		Early	Late	Early		Late		Early		Late	
				1	2	1	2	1	2	1	2
1	I	262	665	510	482	472	458	442	412	314	266
	II	298	635	480	448	442	428	402	384	326	234
2	I	78	208	192	152	112	84	116	96	94	70
	II	62	162	144	136	92	72	98	90	90	66
3	I	246	562	582	564	500	472	468	436	448	440
	II	264	598	540	514	510	498	482	454	460	452
4	I	154	185	166	150	144	136	124	112	112	100
	II	186	225	194	170	112	108	98	86	96	92
5	I	182	312	304	292	240	212	202	186	182	178
	II	168	248	236	228	210	198	178	154	162	158
6	I	196	338	386	305	291	269	268	252	246	226
	II	240	426	325	225	236	204	202	198	188	172
7	I	248	550	478	454	406	364	368	354	346	324
	II	262	362	322	306	256	234	234	228	212	198
8	I	115	202	182	168	132	108	118	102	102	98
	II	95	178	144	138	104	96	98	90	90	78
9	I	305	582	452	418	420	398	390	370	353	321
	II	275	498	392	378	372	322	306	298	282	268
10	I	152	222	198	130	154	150	142	138	132	120
	II	188	310	278	202	228	196	208	192	182	166

from the over-all average data of Table 2. The average level of eosinophils and the average percentile fall in the level during the different phases of the menstrual cycle are shown in Figure 2.

An analysis of the data yielded the following results:

1. The eosinophil counts had maximum values in the late menstrual phase or sometimes in the beginning of the follicular phase.

2. The eosinophil level underwent a progressive fall with the advance-

ment of the follicular phase. The minimum counts for this phase were obtained in the late part covering the period between the tenth and fourteenth days—which time is believed to be the average duration of the ovulatory period. The average fall in the eosinophil count during the early and late parts of the follicular phase was 17 per cent and 33 per cent respectively of the high values obtained for the late menstrual phase.

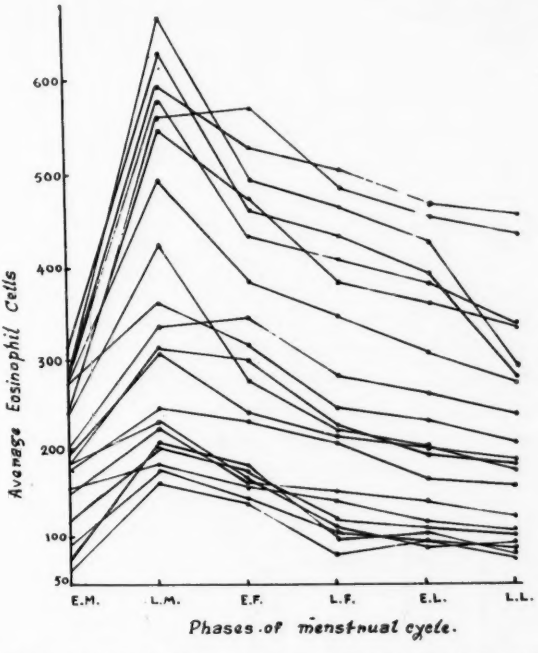

FIG. 1. Pattern of variations in the average number of eosinophils during the different phases of 20 menstrual cycles in 10 women. E.M. = Early menstrual phase; L.M. = Late menstrual phase; E.F. = Early follicular phase; L.F. = Late follicular phase; E.L. = Early luteal phase; L.L. = Late luteal phase.

3. The progressive fall in the level of eosinophils during the follicular phase continued into the luteal phase. The minimum values for the eosinophil count in the luteal phase were obtained toward the end of the phase—a day or two before the actual onset of the menstrual flow. The average total fall in the eosinophil level in the early and late parts of the luteal phase was 38.5 per cent and 45.6 per cent respectively of the high values obtained in the late menstrual phase.

4. During the early part of the menstrual period the results obtained for the eosinophil counts were variable. Whereas in half of the cases (Table

TABLE 2. AVERAGE NUMBER OF EOSINOPHILS PER CUBIC MILLIMETER OF BLOOD DURING VARIOUS PHASES OF A MENSTRUAL CYCLE.

Data obtained by averaging the averages of corresponding phases of the two consecutive menstrual cycles of each subject.

Subject No.	Menstrual phase		Follicular phase		Luteal phase	
	Early	Late	Early	Late	Early	Late
1	280	650	480	450	410	285
2	70	185	156	90	100	80
3	255	580	550	495	460	450
4	170	210	170	125	105	100
5	175	280	265	215	180	170
6	218	382	310	250	230	208
7	255	456	390	315	296	270
8	105	190	158	110	102	92
9	290	540	410	378	341	306
10	170	266	202	182	170	150
Ave.	*198*	*373*	*309*	*261*	*239*	*211*

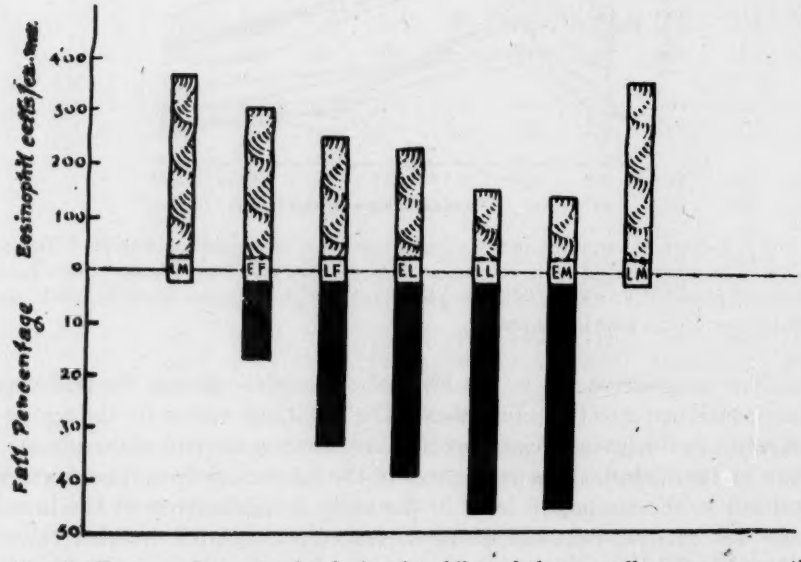

FIG. 2. The over-all average level of eosinophils and the over-all average percentile fall in the eosinophil level during the different phases of the menstrual cycle. (For key to abbreviations *see* legend to Figure 1.)

2, Subjects 4, 5, 6, 8 and 10) there was a rise, in the other half (Table 2, Subjects 1, 2, 3, 7 and 9) there was a further fall. This change, however, was marked only in Subjects No. 3 (decrease) and No. 4 (increase). Thus, during the early part of the menstrual phase, the eosinophil level may either remain low or start rising.

5. The eosinophil level rose in all cases during the late part of the menstrual period and attained a peak either towards the end of this phase or in the beginning of the follicular phase.

DISCUSSION

The level of circulating eosinophils is quite labile and undergoes significant variations even under slightly different conditions. The mental and physical states of the subject, the hour of the day, and the effect of administration of certain drugs, are factors which can bring about noticeable changes in the eosinophil count. The variables influencing the eosinophil level have been reviewed recently (8) by one of us (C.L.P.) It is very important to keep in mind the great lability of the eosinophil level while evaluating the effect of any agent or process on these cells. In our study, due precautions were observed in the selection of cases and the timing of the sampling of blood for conducting the eosinophil cell counts.

Statistical analysis of the data indicates that the values for eosinophil counts obtained during the different phases show a high degree of correlation and highly significant variations. A progressive change is also clearly indicated. *It is therefore evident that significant changes occur consistently* in the level of circulating eosinophil cells during the various phases of a menstrual cycle. The eosinophil level rises during the menstrual phase and falls progressively during the follicular and luteal phases. The whole of the intermenstrual period is characterized by progressive eosinopenia. Thus it is likely that some eosinopenic agent is at work during the intermenstrual phase in increasing intensity, but becomes inactive, inoperative, or is withdrawn during the menstrual phase; whereupon the eosinophil level rises.

The menstrual cycle is controlled through the activity of the ovarian hormones. The concentration of these hormones rises and remains high during the intermenstrual period and falls rather abruptly a few days before the onset of menstruation (9). Thus there seems to be a correlation between the activity of ovarian hormones and the degree of eosinopenia during the intermenstrual period. Evidence of the eosinolytic effect of steroid hormones tends to indicate that most of the steroids except the oxycorticoids are incapable of exerting eosinolytic action (10).

Although it has been emphasized by many workers that the eosinophil level is not a correct guide of adrenocortical function, yet the oxycorticoids

are potent natural eosinolytic agents. It is therefore possible that the changes in the eosinophil level may be due to variations in adrenocortical activity. However, the existence of other factor(s) as the causative agents of the intermenstrual eosinopenia cannot be ruled out.

No definite attempt was made to differentiate between ovulatory and anovulatory cycles. Anovulatory cycles are generally believed to give rise to painless bleeding (12). As some of the menstruations were accompanied with a mild degree of dysmenorrhea, and as the incidence of anovulatory cycles is lower than that of ovulatory cycles, it can be presumed that some of the cycles were ovulatory.

SUMMARY

A study of the level of circulating eosinophil leukocytes was undertaken in order to obtain comprehensive data regarding the level of these cells during the different phases of the menstrual cycle. Eosinophil counts were made by the phloxine technique at 12 noon on two different days during the menstrual period and on eight different days during the intermenstrual period in 10 healthy adult women over a period covering two consecutive menstrual cycles.

It was observed that the level of circulating eosinophils underwent cyclic changes during the different phases of a menstrual cycle. During the whole of the intermenstrual period there was progressive eosinopenia. The eosinophil level was restored to its peak value during the late part of the menstrual phase or soon thereafter.

The activity of the ovarian hormones has been discussed in relation to the eosinophil level.

As the ovarian steroids are not eosinopenic the possibility of variations in adrenocortical activity during the menstrual cycle has been suggested.

Acknowledgments

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PREGNANEDIOL EXCRETION IN NORMAL CHILDREN AND IN CHILDREN WITH VARIOUS ENDOCRINE DISORDERS, INCLUDING CONGENITAL ADRENAL HYPERPLASIA

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THE urinary excretion of pregnanediol has for many years been used as an index of progesterone production in the body. Although progesterone may not be the sole metabolic precursor of pregnanediol—administration of desoxycorticosterone has been shown to cause an increase in pregnanediol excretion—the urinary levels of pregnanediol have been found to bear such a close relation to physiologic events and clinical findings as to leave no doubt that they must be a faithful reflection of progesterone secretion. This is the more surprising when it is considered that, even in late pregnancy, progesterone can barely be detected in blood, and not at all in urine. At this time large quantities of pregnanediol are being excreted. Judging by the conversion of exogenous progesterone, the production of endogenous progesterone should be nearly ten times the urinary excretion of pregnanediol. It is therefore likely that once progesterone is set free in the organism, its existence is so evanescent that the direct measurement of circulating progesterone in peripheral venous blood will not be feasible in nonpregnant subjects however much assay techniques are refined. There is thus every reason to suppose that the assay of pregnanediol will continue to hold a pre-eminent place in the assessment of progesterone metabolism.

Progesterone is produced in at least three organs. Its origin from the ovary and placenta has long been accepted. Pregnanediol has been isolated from male urine (1). Recent observations indicate that there is a small constant production of progesterone from the adrenal in both sexes (2). This work has shown that the level of pregnanediol in the urine reflects the activity of the adrenal, and suggests that the adrenal contribution to urinary pregnanediol represents a secondary catabolic pathway of adrenal

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progesterone splitting off from the main anabolic route by which progesterone is utilized as a building material of other adrenal steroids.

Very few studies have been made of progesterone metabolism in normal children. In children, neither the ovary nor placenta are producing progesterone. Assays of urinary pregnanediol may, therefore, furnish a useful straightforward measure of certain aspects of adrenal function. With this purpose we have made a study of the urinary pregnanediol output of normal children and of those with endocrine abnormalities.

METHODS AND MATERIALS

Methods

For various reasons, no method for assaying pregnanediol in urine has been accepted as entirely satisfactory. Unspecific urinary chromogens have been difficult to separate from pregnanediol and previous methods have usually yielded values which are too high. Klopper, Michie and Brown (3) in 1955 published a method which in several respects seemed superior to earlier procedures. There was good evidence that this method was specific and, on the basis of recovery experiments, had a high degree of accuracy provided the amount of pregnanediol was not too small.

This method of Klopper *et al.* (3) was followed in detail. Due to the low concentration of pregnanediol in the urine of children, the same amount of urine (50 ml.) was used in every determination. Recovery experiments were performed by adding 75 μg. of pregnanediol to 50 ml. of urine before hydrolysis. The average recovery in 10 experiments was 101 per cent, with a range of 80 to 111 per cent.

In an unselected series of 20 different 24-hour urine samples, the average difference found between duplicate readings was 0.07 mg. of pregnanediol per twenty-four hours; the range of difference was 0 to 0.12 mg. Aliquots of the final pregnanediol diacetate obtained from these specimens were pooled and analyzed by infrared spectroscopy. The spectrograms were found to be identical with those of pure pregnanediol diacetate and no contamination with other substances was found.

Subjects

Pregnanediol determinations were made on the urine of 85 "normal" children, 48 girls and 37 boys, from 3 to 15 years old. All the children had been in the hospital for several days and most of them were confined to bed. None showed any signs of metabolic disorders. Some had been admitted to the hospital for social reasons, some were epileptics and some were convalescent from mild acute infections or had minor behavior problems. All the children were afebrile and in good general condition.

In addition, 20 children with various endocrine and metabolic disorders were studied. The diagnoses are shown in Tables 1 and 2.

RESULTS

The results of the pregnanediol determinations in the 85 "normal" children are show in Figure 1. The average value for urinary pregnanediol was 0.73 ± 0.50 (S.D.) mg. per twenty-four hours.

The average value for the 37 boys was 0.76 ± 0.32 mg., and the corresponding value for the 48 girls was 0.72 ± 0.60 mg. per twenty-four hours.

TABLE 1. PREGNANEDIOL EXCRETION IN 14 CHILDREN AND ADOLESCENTS WITH VARIOUS ENDOCRINE AND METABOLIC DISORDERS

Patient	Sex and age (yrs.)	Diagnosis	Pregnanediol excretion (mg./24 hrs.)	Comment
J.B.	M14	Dwarfism (constitutional?)	0.65	
A.S.	F15	Simple goiter	0.85	Postmenstrual phase
S.T.	M13	Pseudohypoparathyroidism	0.53	
H.F.	M15	Obesity; cryptorchidism; hypogenitalism	0.89	
E.S.	M16	Gynecomastia	1.51	
G.W.	M10	Precocious puberty (constitutional type)	1.05	
L.D.	M6	Precocious puberty (tumor cerebri)	0.56	
G.W.	F11	Anorexia nervosa	0.30	Prepubertal
B.O.	F12	Anorexia nervosa	0.90	Amenorrhea for two months
B.S.	F15	Anorexia nervosa	1.19	Amenorrhea for one year
C.v.E.	F14	Metropathia hemorrhagica	0.86	One week after the cessation of bleeding
M.K.	F14	Metropathia hemorrhagica	1.40; 1.44	During the bleeding phase
A.A.	F19	Primary amenorrhea	0.80; 0.86; 1.00	Determinations at weekly intervals
G.W.	F19	Primary amenorrhea	1.05; 1.20; 1.00	Determinations at weekly intervals

As may be seen in Figure 1, there was no definite correlation between the pregnanediol excretion and the age of the children.

In 11 of the 26 girls who were 10 years of age or over the menarche had occurred several months previously (Fig. 2). Eight were in the postmenstrual period or had irregular bleeding. The average pregnanediol excretion in these girls was 0.69 ± 0.20 mg. per twenty-four hours, a value which did not differ significantly from that in the 37 girls who had not reached puberty (0.57 ± 0.57 mg. per twenty-four hours). The average excretion in the 15 girls who were 10 years of age or over but had not yet menstruated was 0.61 ± 0.16 per twenty-four hours. In 3 girls with regular menstrual periods who were in the premenstrual phase when the determinations were made, the values were considerably higher than the other girls (average, 2.56 mg. per twenty-four hours).

TABLE 2. PREGNANEDIOL EXCRETION IN 6 CHILDREN WITH THE ADRENOGENITAL SYNDROME (CONGENITAL ADRENAL HYPERPLASIA): EFFECT OF TREATMENT WITH CORTISONE

Patient	Sex and Age (yrs.)	Pregnanediol excretion (mg./24 hrs.)		Comment
		Before cortisone therapy	During cortisone therapy	
T.S.	M2	8.50	—	
L.U.	M5	—	0.80	
O.Ö.	M6	3.82; 9.15	1.55	Treated with prednisolone
G.M.	F3/12	0.68	0.39	Female pseudohermaphrodism, treated with cortisone
A.H.	F10	10.0	1.48	Female pseudohermaphrodism
N.J.	F15	9.20; 10.2; 9.30; 8.70; 9.60	5.80	Female pseudohermaphrodism

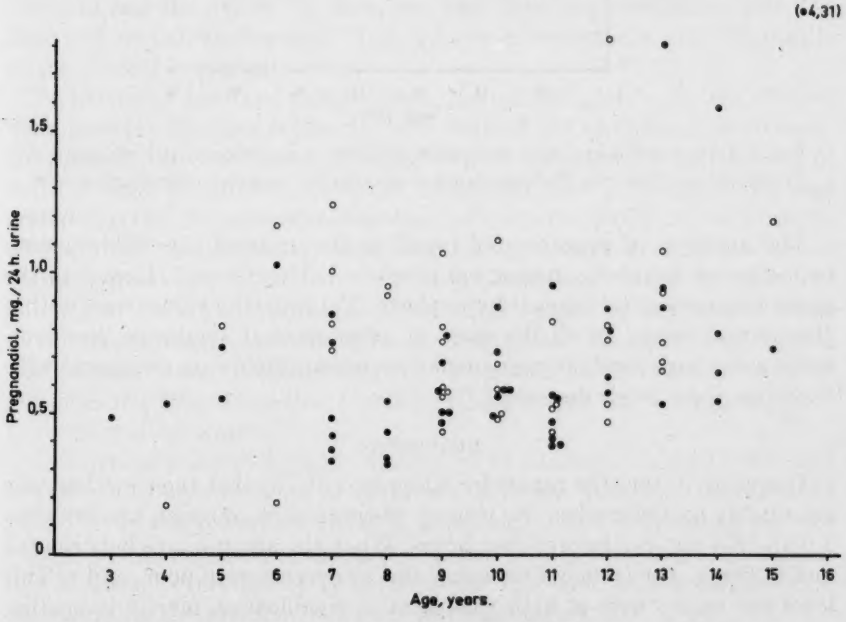

FIG. 1. Daily pregnanediol excretion in 85 normal children, according to age. Girls = •. Boys = o.

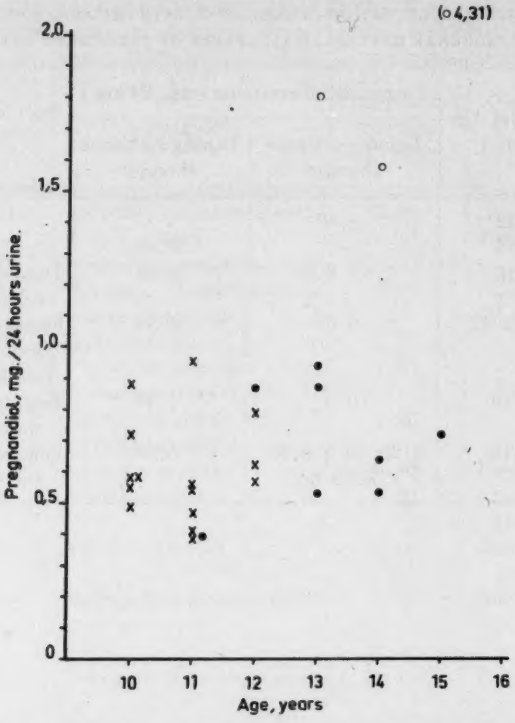

FIG. 2. Pregnanediol excretion in 26 girls 10 years of age or over. Prepubertal=×. Premenstrual phase=o. Postmenstrual phase, having irregular menstruations=•.

The amounts of pregnanediol found in the urine of the children with endocrine or metabolic disease are listed in Tables 1 and 2. Except in the cases with congenital adrenal hyperplasia (Table 2), the values were within the normal range. In all the cases of adrenogenital syndrome, however, there was a high level of pregnanediol excretion. Following treatment with cortisone these levels decreased.

DISCUSSION

It appeared from the report by Klopper *et al.* (3) that their method was reasonably accurate when the urinary pregnanediol excretion was between 1.0 and 0.5 mg. per twenty-four hours. When the amount was between 0.5 and 0.25 mg. per twenty-four hours the recoveries were poor, and at this level the values were of little quantitative significance, merely indicating the presence of traces of pregnanediol. Amounts below 0.25 mg. per twenty-four hours were beyond the sensitivity of the method and could not be

distinguished from zero. In the present study only 2 values were below 0.25 mg. per twenty-four hours (young boys).

The values for pregnanediol excretion in normal men reported by Klopper *et al.* (3) and confirmed by us varied between 0.38 and 1.42 mg. per twenty-four hours, with an average excretion of 0.92 mg. The average excretion in the children of this study was somewhat lower—0.73 mg. Except for the 3 girls in the premenstrual period, the values fell within the range of those for normal adult men.

Because different methods were employed, it is difficult to compare the very few earlier investigations of pregnanediol excretion in children with the results obtained in this study. Talbot *et al.* (4) found no pregnanediol in the urine of normal children. Balassi and Ricca (5) reported increasing pregnanediol excretion with increasing age in a group of 37 girls from 5 to 14 years old. In girls 5 years old or younger, there was no pregnanediol or only faint traces. Six girls who menstruated regularly had the highest values, the maximal excretion being reached between the twenty-third and twenty-fifth days of the menstrual cycle. Veselý (6) assayed the pregnanediol excretion in 19 girls aged 10 to 14 years who had not reached the menarche. He found no correlation between the amount of urinary pregnanediol and the age of the girls, nor was there any correlation with the degree of sexual development. This is more in accordance with the results of the present investigation.

Progesterone has been isolated from the adrenal glands of oxen (7) and is supposed to be a link in the synthetic chain of the adrenocortical steroids. Klopper (8) reported that, following surgical operation or an injection of corticotropin, the urinary excretion of pregnanediol is increased. He suggested that this steroid metabolite reflected the activity of the adrenal cortex which, after stimulation, released progesterone as well as other precursors of the adrenocortical steroids. We have previously shown (9) that the activity of the adrenal cortex in children, as judged by the plasma levels of 17-hydroxycorticosteroids, does not differ from the activity in adults. The present study demonstrates that pregnanediol excretion in children also does not differ from that found by Klopper *et al.* (3) in adult men and postmenopausal women.

Urinary pregnanediol was estimated in 14 children (Table 1) who had symptoms of an endocrine or metabolic disorder. In a boy 16 years old (E.S.) with marked gynecomastia, hypospadias and cryptorchidism the excretion was relatively high (1.51 mg. per twenty-four hours). This boy had a normal excretion of 17-ketosteroids, estrogens and gonadotropins. At operation the presence of testes was established.

In the 6 children with the adrenogenital syndrome (Table 2) there was a very high excretion of pregnanediol, except in the case of the 3-month-old

baby with female pseudohermaphrodism. As no determination was made in a normal infant of this age, it is difficult to state if this level was abnormal. The results of cortisone treatment, however, pointed in this direction. During steroid therapy, pregnanediol excretion was considerably diminished in all these patients.

It has previously been reported (10) that excretion of pregnanediol is increased in congenital adrenal hyperplasia, but normal and subnormal values have been reported by Bierich (11). In 1954 Bongiovanni *et al.* (10) showed that in patients with this disorder a large part of the substance measured as pregnanediol (method of Sommerville and Marrian (12)) was in fact pregnanetriol. Only small amounts of pregnanediol appeared in the urine. These findings naturally raise the question of the specificity of the method used in the present investigation. It is extremely unlikely that the final residues measured by us could have been contaminated with pregnanetriol. The chromatographic properties of pregnanetriol are different from those of pregnanediol and the two steroids are widely separated in both the chromatographic systems in the method we employed.

SUMMARY

Urinary pregnanediol has been estimated by the method of Klopper and associates in 85 children without any known metabolic or endocrine disorders.

The values observed were in good agreement with those found by Klopper *et al.* in adult men and postmenopausal women. The average pregnanediol excretion in boys was 0.75 mg., and that in girls 0.72 mg. per twenty-four hours. For 3 girls in the premenstrual period, the values were higher.

No correlation between pregnanediol excretion and the age of the children could be demonstrated.

In 14 children with various endocrine disorders, values for pregnanediol excretion were essentially normal.

Six children with congenital adrenal hyperplasia were also studied. The high values for urinary pregnanediol before treatment were distinctly reduced during cortisone therapy.

Acknowledgments

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RESPONSE OF THE PLASMA 17-HYDROXYCORTICOSTEROID LEVEL TO GEL-ACTH IN TUMOROUS AND NON-TUMOROUS CUSHING'S SYNDROME

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INTRODUCTION

A KNOWLEDGE of the underlying adrenocortical pathology is of practical importance in the therapeutic management of Cushing's syndrome. There are no clinical signs or symptoms which serve to differentiate Cushing's syndrome due to adrenocortical carcinoma from that due to adenoma or hyperplasia. Differentiation of the adrenal pathology by means of the level of urinary neutral 17-ketosteroids is often impossible, although a very high titer of ketosteroids is suggestive evidence in favor of a malignant adrenocortical lesion (1). Plasma and urinary 17-hydroxycorticoid assays and x-ray examination following retrorectal oxygen insufflation may prove to be indecisive in distinguishing tumorous from nontumorous Cushing's syndrome (1).

More recently, the use of stimulation of the adrenal cortices with corticotropin (ACTH) has been attempted in Cushing's syndrome in an effort to distinguish tumor from hyperplasia (2–4). The few reports in the literature to date concerning the diagnostic usefulness of this method have been encouraging. The changes in the plasma 17-hydroxycorticoid level following the intramuscular administration of Gel-ACTH in 8 cases of classic Cushing's syndrome (including 3 each with hyperplasia and malignant tumor and 2 with adenoma) are reported here. All patients were explored surgically.

METHODS AND CLINICAL MATERIAL

Methods. All patients were studied by the same group of investigators while attending the in-patient service and Endocrine Clinic. The corticotropin (ACTH) test was performed as follows: a 50-cc. sample of venous blood for measurement of the plasma 17-hydroxycorticoid (17-OH-CS) level was collected before, and two hours after the intra-

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muscular administration of 40 units of Gel-ACTH.[1] Studies were made at approximately the same time each morning, without withholding meals. The plasma 17-hydroxycorticoid determinations were performed in duplicate by the method of Silber and Porter (5). In this laboratory, control values for 17-OH-CS in normal subjects range from 3 to 25 gamma per 100 ml. of plasma. We have previously reported that the administration of 40 units of Gel-ACTH to normal subjects results in a maximum increase in the plasma 17-OH-CS level of 14 to 28 gamma per 100 ml., with an over-all average increase of 22 gamma per 100 ml. (6).

The average 24-hour urinary neutral 17-ketosteroid excretion, determined by the method of Holtorff and Koch (7), was 15 mg. in normal females and 10 mg. in normal males. The urinary formaldehydogenic steroids were measured by a modification of the method of Corcoran and Page. Normal values range from 0.5 to 2.0 mg. per twenty-four hours.

Materials. All patients manifested the classic signs and symptoms of Cushing's syndrome, and in each instance there was a significant increase in the urinary formaldehydogenic steroids and the level of plasma 17-hydroxycorticoids. The character of the adrenal lesion was established by surgical exploration. Two patients were found to have an adrenocortical adenoma; and 3, adrenocortical carcinoma. In the remaining 3 patients no tumors were found.

RESULTS

Adrenocortical hyperfunction without tumor

In all 3 patients (Fig. 1A, B and C) the control level of plasma 17-hydroxycorticoids was elevated prior to unilateral adrenalectomy. However, in only 2 patients of this group (L.S. and L.M.) was there an increased responsiveness of the Gel-ACTH test as measured by absolute increments in the plasma 17-OH-CS concentration of 44 and 35 gamma per 100 ml., respectively. Patient M.L. (Fig. 1 B) responded to the test with increases of 9 and 19 gamma per 100 ml. on two occasions preoperatively. In Patient L.M., the test was repeated at one week, one month and three months following surgery. At one month, when there was clinical evidence of a partial remission, a normal control value was observed. However, at three months, coincident with clinical exacerbation, the test values approached the preoperative level. In Patient M.L., eight weeks following unilateral adrenalectomy, normal control values for plasma 17-hydroxycorticoids and a normal response to stimulation with Gel-ACTH were obtained. Evidence of considerable clinical improvement was present at this time.

Adrenocortical adenoma

In both patients (Fig. 2A and B) there was an increase in the control level of plasma 17-hydrocycorticosteroids prior to surgery. In Patient M.F., following the administration of Gel-ACTH, there was a further

[1] Gel-ACTH was generously supplied by Organon, Inc., Orange, N. J.

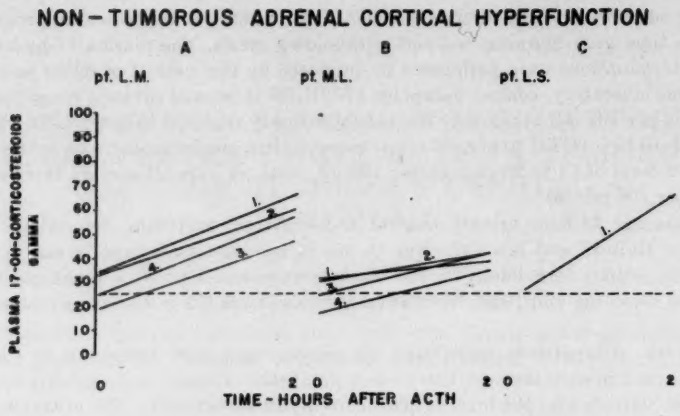

FIG. 1. Plasma 17-OH-CS levels (gamma/100 ml.) **A.** *Curve 1*, preoperative. *Curve 2*, one week after unilateral adrenalectomy. *Curve 3*, one month after operation, *Curve 4*, three months after operation.

B. *Curve 1*, eight weeks before operation. *Curve 2*, two weeks before operation. *Curve 3*, two weeks after unilateral adrenalectomy. *Curve 4*, eight weeks after operation.

C. *Curve 1*, preoperative.

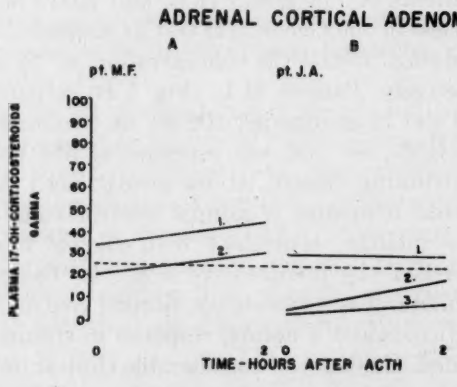

FIG. 2. Plasma 17-OH-CS levels (gamma/100 ml.) **A.** *Curve 1*, preoperative. *Curve 2*, fifty-four days after operation. (Patient received continuous adrenocortical steroid therapy postoperatively, except on days 31–36 and 40–41, and ACTH daily for six days prior to the test.)

B. *Curve 1*, preoperative. *Curve 2*, eighteen days following surgical removal of the tumor (after nine days of ACTH and eighteen days of cortisone). *Curve 3*, thirty-two days following operation (no steroids or ACTH for fourteen days).

modest increase of 12 per 100 ml. In Patient J.A. the preoperative 17-OH-CS level remained unchanged at the end of the test period.

Adrenal carcinoma

The data for these 3 patients are shown in Figure 3. In Patient N.G. (Fig. 3A) with pulmonary metastases from an adrenocortical carcinoma, the level of plasma 17-hydroxycorticoids remained unchanged two hours following the administration of Gel-ACTH.

In Patient A.M. (Fig. 3B) there was an increase in the 17-OH-CS level

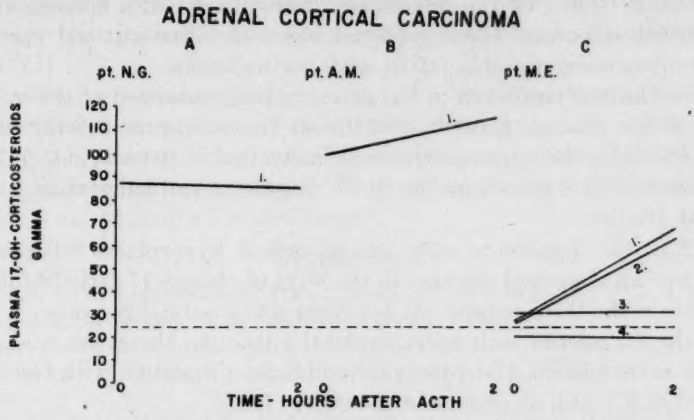

FIG. 3. Plasma 17-OH-CS levels (gamma/100 ml.) **A.** *Curve 1,* three years following surgical removal of tumor; functioning metastases present.

B. *Curve 1,* six weeks after surgical removal of tumor; functioning metastases present.

C. *Curve 1,* preoperative. *Curve 2,* preoperative. *Curve 3,* two days following surgical removal of tumor. *Curve 4,* seven days following operation.

of 19 gamma per 100 ml. following stimulation with the Gel-ACTH.

In Patient M.E. (Fig. 3C), following administration of Gel-ACTH on two occasions preoperatively, there were increases in the plasma 17-OH-CS level of 45 and 42 gamma per 100 ml. above the control values. The patient was prepared for surgery with cortisone, and two days following the removal of carcinoma in the left adrenal the Gel-ACTH test was repeated. Although the level of plasma 17-OH-CS remained elevated, there was now no change following stimulation with Gel-ACTH. Repetition of the test seven days after operation yielded similar results.

DISCUSSION

A review of the literature reveals that the measurement of plasma and urinary 17-hydroxycorticoids following stimulation with corticotropin has

been reported in approximately 25 cases of Cushing's syndrome. In most of the previously reported cases, the changes in the plasma levels or 24-hour urinary titers of 17-OH-CS were determined after periods of intravenous infusion of corticotropin lasting from four to eight hours. Sixteen patients of the group with adrenocortical hyperplasia manifested increased adrenocortical responsiveness, as measured by the plasma and urinary 17-OH-CS (3, 4, 8). In 4 of 6 previously reported instances of adrenocortical adenoma, the pattern of response to the intravenous administration of corticotropin was indistinguishable from that seen in adrenocortical hyperplasia (3, 4, 9, 10). Of the remaining 2 patients, 1 had a normal, and 1 a diminished response. Three reported cases of adrenocortical carcinoma were unresponsive to stimulation with corticotropin.

The technique employed in the present study consisted of the measurement of the plasma 17-hydroxycorticoid concentration before, and two hours following the intramuscular administration of 40 units of Gel-ACTH. The merit of this procedure lies in its simplicity and adaptability to outpatient studies.

In 2 of the 3 patients with adrenocortical hyperplasia reported here there was an abnormal increase in the level of plasma 17-OH-CS following the test; in the third patient (M.L.) there was a normal response.

Of the 2 patients with adrenocortical adenoma, there was a slight response of the plasma 17-hydroxycorticoids to stimulation with Gel-ACTH in one (M.F.), and no change in the other (J.A.).

In 2 of the 3 patients with adrenocortical carcinoma, little or no change occurred in the plasma 17-hydroxycorticoid level following the administration of corticotropin. However, the third patient responded quite differently. On two occasions prior to the surgical removal of the carcinoma, there were sharp rises in the plasma level of 17-OH-CS following administration of Gel-ACTH. Quantitatively, these changes were of an order similar to that seen in 2 of our patients with adrenocortical hyperplasia. The responsiveness of the malignant tumor to corticotropin in this instance was demonstrated by the postoperative results, *i.e.*, by the failure of the plasma 17-OH-CS level to increase following the administration of Gel-ACTH, by the second or even by the seventh day after removal of the tumor. A case of adrenocortical carcinoma sensitive to stimulation with corticotropin has not been previously reported.

In 5 patients, Gel-ACTH tests were employed at varying intervals after surgery, to determine the status of the remaining adrenal tissue.

SUMMARY

1. A study is presented of the changes in the level of plasma 17-hydroxycorticoids following the intramuscular administration of Gel-ACTH in 8

patients with Cushing's syndrome due either to tumorous or non-tumorous adrenocortical hyperfunction.

2. The preoperative tests revealed that in 2 of the 3 patients with non-tumorous adrenocortical hyperfunction there was an increased responsiveness to Gel-ACTH; in the third patient the response was normal. In the 2 cases of adrenocortical adenoma, there was a diminished response to the corticotropin in one, and complete failure of response in the other. In 2 of the 3 patients with adrenocortical carcinoma there was either a decreased response or no change; in the third, the level of plasma 17-hydroxycorticoids was abnormally increased. Subsequent studies showed that this increase was due to the responsiveness of the malignant tumor to stimulation with Gel-ACTH.

3. On the basis of these studies, it may be concluded that in Cushing's syndrome the presence of an adrenocortical tumor is strongly suggested by failure of the plasma 17-hydroxycorticoid level to increase following the administration of gel-corticotropin. The presence of a normal or augmented response is not of diagnostic significance.

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EFFECT OF THYROID THERAPY ON THE TONE AND CONTRACTILITY OF THE URINARY BLADDER IN HYPOTHYROIDISM

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INTRODUCTION

IT IS a well known fact that in myxedema there is hypotonia of the heart (1), of the uterus (2), of the alimentary canal (manifested as megaduodenum (3) or megacolon (4, 5)), and of the gallbladder (6, 7).

Evans (8) mentions the case of a myxedematous patient who had difficulty at the beginning of micturition and pollakiuria, and in whom volumes of residual urine ranging from 700 to 1,500 cc. were obtained by catheterization of the bladder. A similar case was observed by Escamilla (9).

All these manifestations of hypotonia of the visceral muscles disappear after treatment with desiccated thyroid.

These facts led us to make a graphic study of the tone of the urinary bladder in myxedema, employing the method which Alvarez and Caldeyro-Barcia (10) developed for the study of the tone of the pregnant uterus.

In this report we present the results obtained in 3 patients with primary thyroid insufficiency.

METHOD

To determine the tone and contractility of the bladder, the intravesical pressure was recorded (Fig. 1). For this purpose, a water manometer is connected to the vesical cavity by means of a catheter introduced through the urethra. The manometer is provided with a recording device which inscribes the vesical pressure upon a kymograph.

The record is begun with the bladder nearly empty (contents 20 cc.). The bladder continuously registers small, rhythmic contractions of 1 to 3 cm. in amplitude. We consider "tone" to be the pressure exerted by the bladder between the contractions (Fig. 2). In order to measure the "tone," the intraperitoneal pressure is considered as the zero point on our scale, in accordance with the bases and methods published by Duomarco and Rimini (11), and by Alvarez and Caldeyro-Barcia (10).

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Intravesical injections of 50 or 100 cc. of sterile physiologic saline are made by means of a syringe, through the catheter used for recording the intravesical pressure. During each injection, the record is interrupted by means of turning a three-way stop-cock. After each injection the contractility is recorded for ten to twenty minutes, in order to determine the influence of distention upon vesical tone and upon the amplitude of the contractions of the bladder.

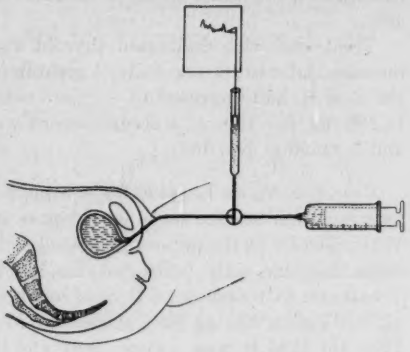

FIG. 1. Method for recording intravesical pressure.

The intravesical injections are continued until a pressing desire to micturate is felt by the patient. At this moment the vesical distention curve is finished. The vesical evacuation curve is then recorded by means of successive removals of 100 cc. of liquid at a time, the vesical contractility being registered for ten to twenty minutes after each extraction.

CLINICAL MATERIAL

Case 1. V.R. de M. (#14869), a woman aged 42 years, had typical primary myxedema, with symptoms starting three years before. When seen by us, the patient presented in-

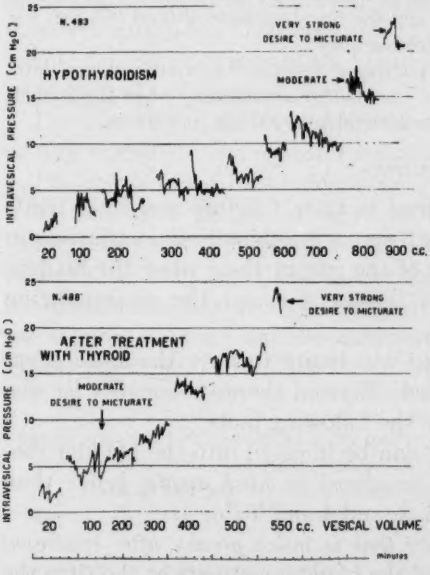
N. 483
VERY STRONG DESIRE TO MICTURATE
HYPOTHYROIDISM
MODERATE
INTRAVESICAL PRESSURE (Cm H2O)
20 100 200 300 400 500 600 700 800 900 cc.
N. 488
VERY STRONG DESIRE TO MICTURATE
AFTER TREATMENT WITH THYROID
MODERATE DESIRE TO MICTURATE
20 100 200 300 400 500 550 c.c. VESICAL VOLUME
minutes

FIG. 2. Tracings of intravesical pressure during bladder distention in hypothyroidism, before and after treatment with desiccated thyroid (Case 1). The breaks in the recording correspond to the injection of physiologic saline into the bladder. Note the decrease in distensibility after treatment.

filtrated skin, puffy features, hoarseness and loss of axillary hair. She was apathetic and suffered from the cold. No treatment had been given. On September 1, 1954, the B.M.R. was −15 per cent, and the blood cholesterol level 300 mg. per 100 cc. Under these cir-

cumstances, the initial record of bladder function was made (Figs. 2 and 3, tracings No. 483).

Treatment with desiccated thyroid was then begun in a dosage of 50 mg. daily, increased later to 100 mg. daily. A great improvement in clinical symptoms ensued. When the B.M.R. had increased to −2 per cent and the blood cholesterol level had dropped to 190 mg. per 100 cc., a second record was made of the bladder contractility (Figs. 2 and 3, tracings No. 488).

Case 2. E.M. de L. (#14224), a woman aged 24 years, had primary myxedema. The symptoms had started seven years before and became typical after four years and a half. When seen by us the patient presented asthenia, apathy, mental sluggishness, puffy features, cold, dry, scaly, infiltrated skin, hoarseness, constipation, and sensitiveness to cold. Treatment with desiccated thyroid had been followed sporadically for a year and a half, all medication having been suspended four months before consultation. On August 6, 1954, the B.M.R. was −24 per cent, and the blood cholesterol level 455 mg. per 100 cc. Under these circumstances a record was made of bladder contractility (Fig. 4, tracing No. 498).

Treatment was then begun with 100 mg. per day of desiccated thyroid. This dosage was continued until the disappearance of clinical symptoms. The B.M.R. was +17 per cent, and the blood cholesterol level 203 mg. per 100 cc. A new record of bladder contractility was then made (Fig. 4, tracing No. 513).

Case 3. M.E.R. (#14780), a woman aged 22 years, had primary myxedema of three years' duration, which had come on gradually, with dry, rough, infiltrated skin, puffy features, loss of hair, asthenia, constipation, hoarseness, dry hair and sensitiveness to the cold. The B.M.R. was −47 per cent, and the blood cholesterol level 305 mg. per 100 cc. A record was made of the bladder contractility.

Treatment was then started with 50 mg. per day of desiccated thyroid, increased later to 100 mg. daily. A new record was made of the bladder contractility when the B.M.R. had reached +10 per cent, and the blood cholesterol level 137 mg. per 100 cc.

RESULTS

Figure 2 shows the tracings obtained in Case 1 before and after treatment with desiccated thyroid. Figure 3 shows graphically the variations in the vesical tone and in the intensity of the contractions when the bladder was gradually distended, in Case 1. Figure 4 shows the corresponding findings in Case 2.

Treatment with desiccated thyroid was found to have the same effect on bladder tone in the 3 cases studied. Thyroid therapy *decreases the distensibility of the bladder*, as shown by the following facts:

1) The *total amount of fluid* which can be injected into the bladder (before a pressing desire to micturate develops) is *much greater before* than after treatment, as seen in Figures 2, 3, and 4 and Table 1.

2) For equivalent volumes, *bladder tone is much greater after treatment* than before. The larger the volume of the bladder contents at the time the comparison is made, the greater the difference in tone. For instance in Case 2, for a volume of 50 cc., the tone registered 6 cm. of water before treat-

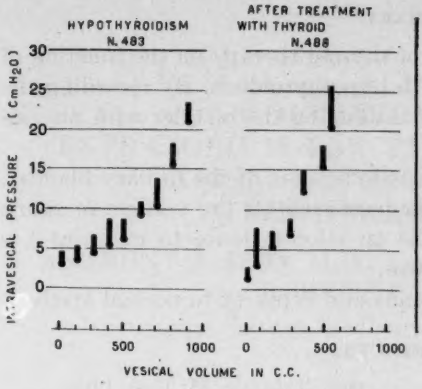

FIG. 3

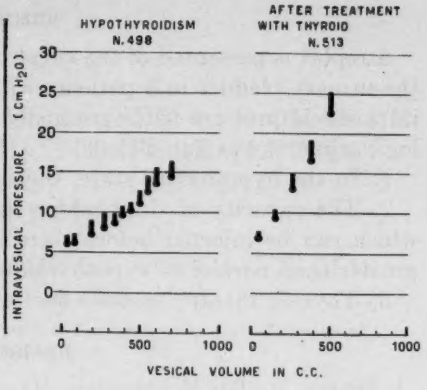

FIG. 4

FIG. 3. Graphic representation of the values for vesical tone (solid black dots) and for the intensity of the rhythmical contractions (height of bars) during gradual distention of the bladder in hypothyroidism (Case 1). Note that the curve is much steeper after thyroid therapy.

FIG. 4. Corresponding values in Case 2.

ment and 7 cm. after treatment; whereas, for a volume of 500 cc., treatment raised the tone reading from 11 cm. to 22 cm. of water (Fig. 4). As a result, the curve representing bladder distention was much steeper after treatment than before.

The intensity of the rhythmic contractions of the bladder did not undergo any significant change after treatment with desiccated thyroid.

The values found for bladder tone in these cases of hypothyroidism after treatment with desiccated thyroid were similar to those reported by Denny-Brown and Robertson (12) for normal people. As the values for vesical tone were lower before treatment, it is evident that hypotonia of the bladder accompanies hypothyroidism. This finding is in agreement with the reports of other authors concerning hypotonia of various viscera in hypothyroidism.

TABLE 1. EFFECT OF TREATMENT WITH DESICCATED THYROID UPON THE URINARY BLADDER IN TERMS OF THE MAXIMUM VOLUME OF PHYSIOLOGIC SALINE WHICH COULD BE INJECTED BEFORE THERE WAS A PRESSING DESIRE TO MICTURATE

	Case 1	Case 2	Case 3
Before treatment	700 cc.	900 cc.	820 cc.
After treatment	500 cc.	580 cc.	690 cc.

SUMMARY

A report is presented of the effect of thyroid therapy on the function of the urinary bladder in 3 patients with hypothyroidism. By recording the intravesical pressure while gradually distending the bladder with physiologic saline, it was found that:

1. In the hypothyroid state, there is hypotonia of the urinary bladder.
2. The capacity of the bladder (as measured by the volume of saline which can be injected before there is an intense desire to micturate) is greater than normal in hypothyroidism.
3. Thyroid therapy restores the tonus and capacity to normal levels.

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SPECIAL CONSIDERATIONS IN THE USE OF BLUE TETRAZOLIUM FOR THE MEASUREMENT OF ALPHA-KETOLS IN MIXTURES WITH OTHER STEROIDS*

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INTRODUCTION

A VARIETY of chemical methods has been proposed for the quantitative estimation of adrenocortical steroids (1–9). Several of these depend on the presence of the alpha-ketol side-chain, a grouping on the molecule characteristic of the active adrenocortical steroids. Selective reduction of tetrazolium salts by this grouping has been shown to occur under mild conditions (10). Chen and Tewell (8, 9) and later Mader and Buck (11) used blue tetrazolium (3,3′-dianisole-bis-4,4′-3,4 diphenyltetrazolium chloride) for the quantitative estimation of these steroids.

These investigators have defined many of the essentials of the procedure, such as the absorption maximum of the chromogen, the adherence to Beer's law, and the advantages of tetramethyl-ammonium hydroxide as base over choline or sodium hydroxide. There is, however, disturbing uncertainty in regard to several factors which may imperil the applicability of the procedure to certain biologic mixtures. Although all investigators agree that ketols react strongly with blue tetrazolium (BT), there is some disagreement regarding precise conditions for maximum color and reproducibility, and it has been questioned whether all ketols react equally. Furthermore certain non-ketols possessing a conjugated keto group have been reported to be reactive under some conditions (9). An extensive study of this problem was recently reported by Meyer *et al.* (12). These authors had in mind the accentuation of differences in reducing powers among various steroids for purposes of identification.

Our study was undertaken with a somewhat different aim. We, also, were interested in the extent to which such variables as concentration of base,

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time, temperature of reaction, and purity of solvents affected the reactivity of various steroids. The purpose, however, was to determine conditions which minimized or allowed correction for the reduction of non-ketolic steroids so that alpha-ketols could be measured with accuracy when in mixtures containing non-ketolic steroids.

With the modification we adopted, abundant and equal color is achieved with all ketolic steroids. Of the non-ketols, only those possessing a Δ^4,3-keto group caused reduction of BT, but they produced less color and at slower rates.

EXPERIMENTAL

Reagents and solutions

1. Blue tetrazolium (BT), DAJAC Laboratories, m.p. 242–243° C.—0.25 per cent solution in purified ethanol (*see* Item 5).

2. Tetramethyl-ammonium hydroxide (Distillation Products, Inc.)—10 per cent in water. Dilutions were made with purified ethanol to the concentrations described in the experiments which follow. The dilute solutions were made shortly before use.

3. Acid alcohol. Concentrated hydrochloric acid was diluted 1:10 with redistilled ethanol. This solution is stable for months.

4. Solutions of pure steroids in methanol.

5. Purified ethanol. The following methods were used with equal success. (The alcohol so prepared and kept in brown glass bottles was found stable for two weeks; after that the values for the reagent blanks increased rapidly.)

a) Metaphenylene-diamine dihydrochloride (2 Gm./100 ml.) was added to the alcohol; the mixture was shaken, stoppered and allowed to stand in the dark for periods up to three months. The solution turned a purplish color. The alcohol was distilled slowly with a fractionating column, discarding the head and tail fractions.

b) Ethyl alcohol (95 per cent) was distilled over sodium hydroxide pellets; 2 per cent of metaphenylene-diamine dihydrochloride was added to the distillate and refluxed several hours. Redistillation was then carried on as in procedure "a."

c) Method described by Fieser. Sodium metal weighing 7 Gm. was added in small portions to 1 liter of alcohol, followed by the addition of 27.5 Gm. of diethyl phthalate. The mixture was refluxed for one hour protected from moisture, and was then distilled in the manner just described.

Method

1. Measured quantities of pure steroids were transferred to glass-stoppered test tubes, and the solvent evaporated off in a water bath of 45° C. with the aid of a stream of nitrogen.

2. The steroid was redissolved by addition of 3.0 ml. of purified ethanol.

3. 1.0 ml. of the 0.25 per cent BT solution was added.

4. The tubes were immersed in a water bath of 25° C. and the reaction was started by addition of 1.0 ml. of the dilute tetramethyl-ammonium hydroxide.

5. 1.0 ml. of acid alcohol was added at the proper time to stop the reaction.

A reagent blank was prepared by substituting alcohol for the steroid solution, and a reverse blank by the addition of the acid alcohol before the tetramethyl-ammonium hydroxide to prevent any reaction in the blank.

A Coleman #14 special spectrophotometer set at 510 millimicrons was used. The results were the same with a Beckman spectrophotometer. The Coleman #14 instrument permits more rapid work. The reagent blanks were read against the reverse reagent blanks and the steroid solutions against the reagent blanks.

RESULTS

The effect of varying the strength of tetramethyl-ammonium hydroxide (T.M.A.H.) reagent from 0.1 per cent to 10.0 per cent was first tested. This resulted in concentrations of 0.02 per cent (0.0022 N) to 2.0 per cent (0.22 N) in the final reaction mixtures. All reference hereafter will be to the concentration of this base in the reagent. Eleven alpha-ketols and four non-ketolic steroids with conjugated 3-keto groups were first tested in this manner. The pertinent findings are recorded in Figure 1. On the basis of the results, which will be discussed, a T.M.A.H. concentration of 1 per cent was considered optimal, and series of determinations were performed on 40 compounds. These are recorded in Table 1.

In the case of the ketolic steroids with conjugated 3-keto groups, maximum color was attained at all alkali concentrations between 0.2 and 2.0 per cent. At the extreme concentrations, 0.1 and 10.0 per cent respectively, maximum color was never attained. With the highest alkali concentrations color was actually lost, as the reaction time was prolonged beyond fifteen minutes.

Most of the ketolic steroids saturated in ring A reacted at slower rates with concentrations of T.M.A.H. reagent less than 1.0 per cent (Fig. 1). At these lower concentrations the compounds with an 11-keto group which we were able to study developed maximum color faster than those with 11-hydroxy groups. These variations among all the ketols were minimal and probably not significant when the alkali reagent contained 1.0 per cent T.M.A.H. and the reaction was stopped at thirty or sixty minutes, as may be seen in Figure 2 and in Table 1, compounds 1 to 11. Compounds 12 and 13 did not behave in this manner, but they are not naturally occurring steroids.

The non-ketolic compounds causing reduction were those steroids possessing a Δ^4,3-keto group (Table 1, compounds 22 to 32; and Fig. 1). Instead of reacting rapidly and reaching a plateau in the manner of the ketols, they reacted at a steady but much slower rate, especially when the alkali concentration was low (testosterone in Fig. 1). At 1.0 per cent T.M.A.H., the rate of reaction was a straight-line function for the first sixty minutes in each instance. When readings were made at both thirty and sixty minutes, therefore, correction for large amounts of such non-ketolic steroids could be made (Table 2). This could not be done when the concentration of alkali was 2 per cent.

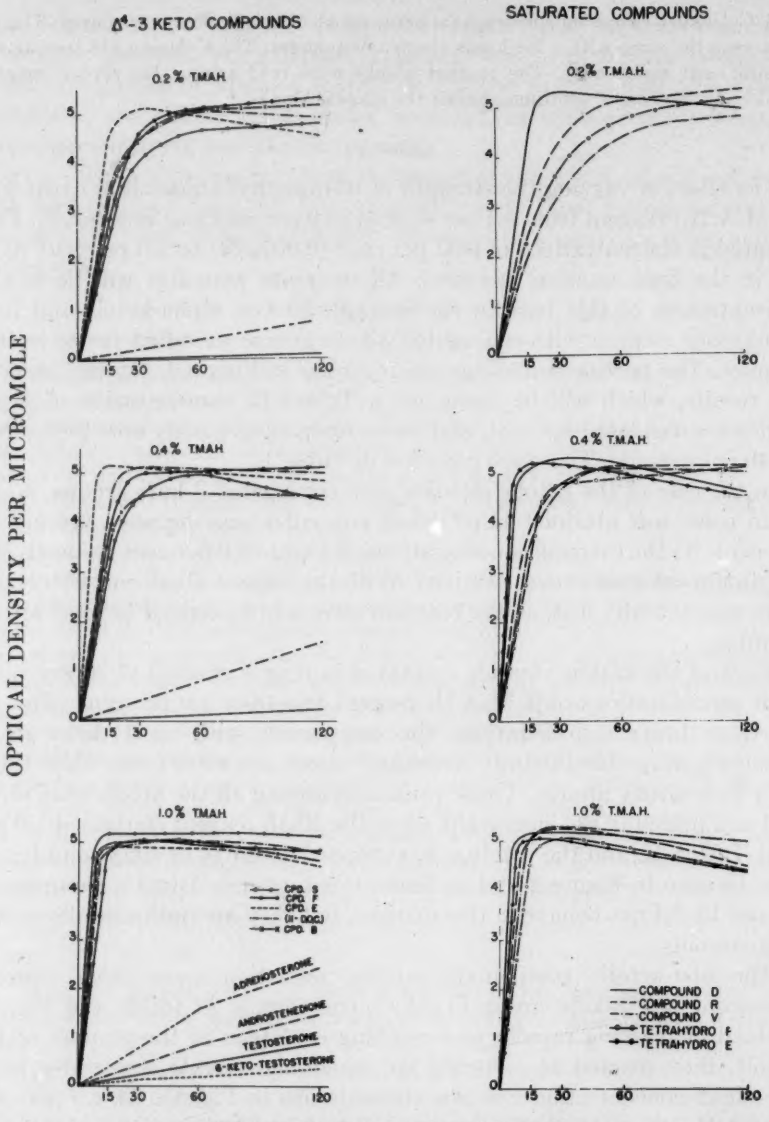

FIG. 1. The reduction of blue tetrazolium by various steroids in the presence of different concentrations of tetramethyl-ammonium hydroxide reagent. In the left column are Δ^4,3-keto compounds; in the right column are saturated alpha-ketols. Experiments with lesser and greater concentrations of alkali are not recorded but are described in the text.

TABLE 1. OPTICAL DENSITIES OBTAINED WHEN BLUE TETRAZOLIUM WAS REDUCED BY VARIOUS STEROIDS IN PRESENCE OF 1 PER CENT T.M.A.H. REAGENT

Steroid	No. of det'ns	Micrograms in aliquot	Calculated optical density per micromole			
			Reaction time			
			15 min.	30 min.	60 min.	120 min.
1. 11-Desoxycorticosterone	6	32	4.82 ± .23	4.97 ± .23	5.04 ± .28	4.77 ± .20
2. 11-Desoxy,17-hydroxycorticosterone	8	16–40	5.21 ± .22	5.15 ± .05	5.25 ± .21	5.13 ± .29
3. Corticosterone	4	35	4.96 ± .26	5.03 ± .27	5.02 ± .12	4.63 ± .14
4. 11-Dehydrocorticosterone	2	25	5.21 ± .08	5.12 ± .05	5.20 ± .07	5.48 ± .06
5. 11-Dehydro,17-hydroxycorticosterone (cortisone)	6	20–31	4.91 ± .07	4.90 ± .28	4.65 ± .15	4.48 ± .09
6. 17-Hydroxycorticosterone (hydrocortisone)	6	35	4.81 ± .04	5.04 ± .13	4.96 ± .10	4.80 ± .11
7. Pregnane,3-alpha,17-alpha,21-triol,11,20-dione (tetrahydro E)	4	22–43	5.07 ± .03	4.90 ± .10	4.63 ± .10	4.31 ± .11
8. Pregnane,3-alpha.11-beta, 17-alpha,21-tetrol,20-one(tetrahydro F)	2	24	4.51 ± .02	5.03 ± .00	4.67 ± .02	4.38 ± .06
9. Allopregnane,3-beta,11-beta,21-triol,20-one (compound R)	4	28	5.04 ± .08	5.30 ± .05	5.09 ± .07	4.94 ± .52
10. Allopregnane,3-beta,17-alpha,21-triol,11,20-dione (compound D)	6	26	5.18 ± .02	4.84 ± .09	4.69 ± .01	4.41 ± .03
11. Allopregnane,3-beta,11-beta,17-alpha,21-tetrol,20-one (compound V)	2	25	4.99 ± .06	5.26 ± .02	5.19 ± .04	4.99 ± .04
12. 9-Alpha-fluorohydrocortisone	4	20–40	4.90 ± .02	4.95 ± .09	4.21 ± .03	3.69 ± .09
13. Δ',4-Pregnadiene,17-alpha,21-diol,3,11,20-trione (metacortandracin)	5	20–40	4.06 ± .11	4.32 ± .37	3.84 ± .33	3.07 ± .12
14. Pregnane,3-alpha-ol,20-one	2	192	0.012 ± .004	0.012	0.027 ± .006	0.036 ± .003
15. Pregnane,3-alpha,12-alpha-diol, 20-one	2	114	0.016 ± .007	0.012 ± .012	0.009 ± .009	0.042 ± .005
16. Pregnane, 3,20-dione	4	160–208	0.028 ± .008	0.040 ± .007	.075 ± .012	.089 ± .018
17. Pregnane,3,12,20-trione	2	100	0.026 ± .002	0.033 ± .004	.035 ± .006	.086 ± .006
18. Pregnane,3-alpha.17-alpha-diol, 11,20-dione	2	120	0.003 ± .002	0	0	0
19. Allopregnane,3-beta,17-alpha-diol,20-one	2	150	0.014 ± .006	0	0	0
20. Pregnane,3-alpha,17-alpha,20-triol	3	144	0.035 ± .006	0.037 ± 0	0.079 ± .020	0.042 ± .012
21. Pregnane,3-alpha,17-alpha,20, 21-tetrol,11-one	3	168	0	0.024 ± 0	0.041 ± .007	0.188 ± 0
22. Δ⁴,Pregnene,3,20-dione (progesterone)	4	212	0.152 ± .030	0.276 ± .042	0.541 ± .066	0.921 ± .015
23. Δ⁴,Pregnene,17-alpha-ol,3,20-dione (17-hydroxyprogesterone)	2	120	0.124 ± .005	0.241 ± .008	0.454 ± .008	0.778 ± .005
24. Δ⁴,Pregnene,6-beta-17-alpha-diol,3,20 dione (6,17-dihydroxyprogesterone)	2	130	0.034 ± .002	0.042 ± .004	0.114 ± .004	0.182 ± 0
25. Δ⁴,Pregnene,17-alpha,20,21-triol,3-one	2	104	0.117 ± .003	0.210 ± .007	0.337 ± .010	0.620 ± .002
26. Δ⁴,Pregnene,17-beta,20,21-triol, 3-one	2	100	0.100 ± .009	0.195 ± .009	0.340 ± .0	0.580 ± .004
27. Δ⁴,Pregnene,11-beta,17-alpha, 20,21-tetrol,3-one (Reichstein's cpd. E)	3	170	0.055 ± .008	0.092 ± .002	0.169 ± .002	0.319 ± .006
28. Δ⁴,Pregnene,17-alpha,20,21-triol,3,11-dione (Reichstein's cpd. U)	3	196	0.183 ± .002	0.375 ± .004	0.682 ± .010	1.455 ± .004
29. Δ⁴,Androstene,17-beta-ol,3-one (testosterone)	4	245–290	0.113 ± .028	0.224 ± .048	0.456 ± .058	0.883 ± .088
30. Δ⁴,Androstene,17-beta-ol,3,6-dione (6-keto-testosterone)	2	48	0.088 ± 0	.151 ± .025	.189	.359 ± .020
31. Δ⁴,Androstene 3,17-dione	2	132	0.227 ± .007	0.403 ± .002	0.843 ± .008	1.47 ± 0
32. Δ⁴,Androstene,3,11,17-trione (adrenosterone)	3	192	0.408 ± .004	0.819 ± .019	1.58 ± 0	2.43 ± .015
33. Δ⁵,Androstene,3-beta,17-alpha-diol	2	200	0.001 ± 0	0.004 ± .004	0.004 ± .007	0.007 ± .003
34. Androstane,17-one	2	200	0.015 ± .001	0.009 ± .005	0.000 ± .002	0.007 ± .005
35. Androstane,3-alpha-ol,17-one (androsterone)	2	208	0.005 ± 0	0.003 ± 0	0.002 ± .001	0.001 ± .001
36. Androstane,17-alpha-ol,3-one	2	212	0.022 ± .002	0.031 ± .002	0.035 ± .002	0.057 ± .000
37. Androstane,3-alpha,17-alpha-diol	2	200	0.007 ± .004	0.010 ± .003	0.001 ± .002	0
38. Androstane,3,17-dione	2	200	0.019 ± .005	0.016 ± .006	0.039 ± .006	0.097 ± .016
39. Etiocholane,3-alpha-ol,11,17-dione	2	208	0.010 ± .004	0.021 ± .003	0.010 ± .002	0.018 ± .002
40. Cholesterol	2	416	0.032 ± .020	0.006 ± .000	0.006 ± .006	0

Additional considerations which influenced the selection of the best conditions for the quantitative measurement of ketols are illustrated in Figure 2. It should again be noted that optimum conditions were different for each group of BT-reducing substances. It should also be emphasized again that it was of importance to select conditions that would be favor-

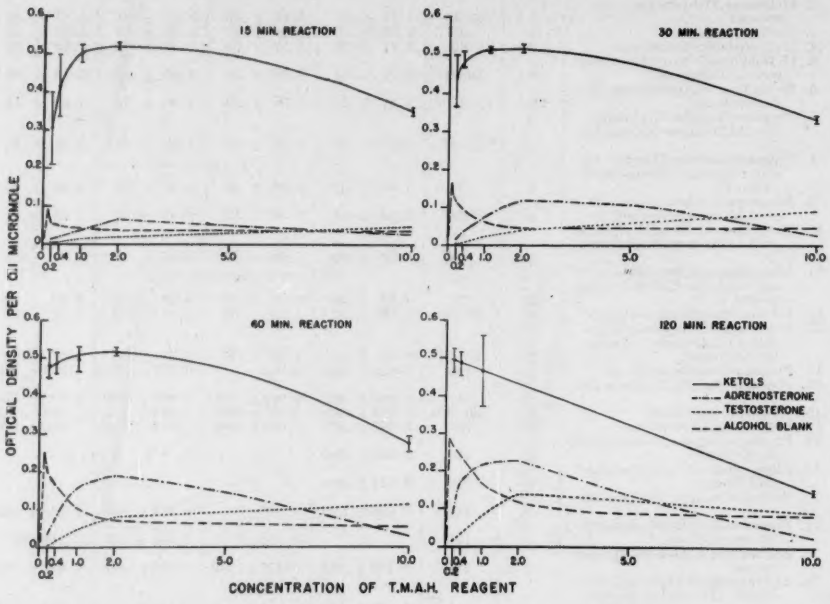

FIG. 2. Summary of data from which the optimum conditions for the BT reaction were selected. Each graph represents the time at which the reaction was stopped for all the reagent concentrations tested.

The alpha-ketols are represented by the solid lines and the vertical lines which cross at various points indicate one standard deviation from the mean for all ketols studied at that particular concentration.

able for the tetrahydro compounds as well as the biologically active cortical steroids, since they are the major ketols in some biologic mixtures, especially urine. The greater standard deviations at low concentrations of T.M.A.H. were due in greatest part to the slower reactivity of such saturated compounds. The concentration of alkali and the time of reaction were of considerable importance even in the alcohol blank. The latter formed maximum color when T.M.A.H. concentrations of 0.1 per cent and 0.2 per cent were used. The conditions selected as most favorable for accurate determination of ketols were: a thirty-minute reaction time and a

1 per cent concentration of T.M.A.H. reagent. Under these conditions the optical density of the blank was 0.064 (S.D. ±0.011).

Several items appearing in Table 1 are of interest. In the case of the non-ketols with alpha-beta unsaturation, an additional ketone group at the 11 or 17 carbon increased the rate of reaction, and a third ketone at one of these positions speeded it up even more (compare compound 27 with 28, Table 1; and compounds 29, 31 and 32, Table 1 and Fig. 1). Interestingly enough, however, the additional ketone group at C-6 in 6-keto-testosterone

TABLE 2. OPTICAL DENSITIES OBTAINED WHEN BLUE TETRAZOLIUM WAS REDUCED BY ALPHA-KETOLS IN THE PRESENCE OF SMALL AMOUNTS OF ADRENOSTERONE

(The optical densities produced by the mixtures are compared with the sums of the densities produced separately.)

Compound	Micrograms used	Optical density at		
		30 min.	60 min.	120 min.
1. Cortisone	53.4	.787 ± .005	.757 ± .005	.752 ± 0
2. Adrenosterone	11.5	.024 ± .006	.048 ± .008	.060 ± .004
3. Tetrahydrocortisone	42.3	.572 ± .015	.552 ± .010	.515 ± .013
1 & 2. Mixture	53.4+11.5	.800 ± 0	.812 ± 0	.792 ± 0
Difference of mixture density from sum of densities of 1 & 2		−1.1%	+0.9%	−2.4%
2 & 3. Mixture	11.5+42.3	.602 ± .005	.605 ± .013	.547 ± 0
Difference of mixture density from sum of densities 2 & 3		+1.0%	+0.8%	−4.9%

decreased the rate of reduction from that exhibited by testosterone (compare compounds 29 and 30, Table 1 and Fig. 1). The addition of a 6-beta-hydroxyl group at this position had a similar influence on the reactivity of 17-hydroxyprogesterone (compare compounds 23 and 24, Table 1).

Compounds with a Δ^5,3-hydroxyl configuration, such as cholesterol (No. 40) and Δ^5,androstenediol (No. 33) produced no color at thirty minutes or later.

Neither ketone groups at position 17, nor alcohol groups at position 3 or 17 produced color when the steroid lacked other reactive groups. The 20-ketone groups, non-conjugated 3-ketone groups, 17,20 "diols" (No. 20), and 17,20,21"triols" (No. 21) contributed barely measurable optical densities, probably of no significance in the measurement of mixtures containing alpha-ketols.

Having decided on the most favorable conditions for the reaction, we

were interested in determining whether the reduction of BT by alpha-ketols and the more slowly reducing non-ketols is the same, whether in mixtures and separately. It was necessary to examine several sets of conditions.

In Table 2 are listed the optical densities obtained with reaction mixtures containing alpha-ketols (with and without alpha-beta unsaturation in ring A) and relatively small amounts of adrenosterone, the most reactive non-ketolic Δ^4,3-keto compound in our possession. The reducing powers of the steroids separately and in combination were studied simultaneously. It was found that optical densities obtained with the mixtures were essentially the same as the sums of the separate densities, especially at the thirty-minute and sixty-minute reaction times.

When larger amounts of the non-ketolic, slowly reacting, reducing steroids were added to cortisone or tetrahydrocortisone it was found again that there was good agreement between the arithmetic sums of the separate optical densities and the optical densities of mixtures of the same steroids.

In each of these mixtures the item particularly pertinent to the quantita-

TABLE 3. ESTIMATION OF THE AMOUNT OF ALPHA-KETOLS IN MIXTURES CONTAINING STEROIDS WHICH REDUCE BT SLOWLY

Mixture		Increase of O.D.* between 30 and 60 min. attributable to non-ketols		O.D. at 30 min. attributable to alpha-ketols		Alpha ketols in mixture (μg.)		
Compounds	Amount (μg.)	Increase observed in mixture	Expected from non-ketols alone	Mixture corrected for observed non-ketol increment	Expected from ketols alone	Known to be present	Found (corrected)	Found (uncorrected)
Cortisone Adrenosterone	53.4 11.5	0.012	0.024	0.788	0.787±0.005	53.4	53.6	54.6
Tetrahydrocortisone Adrenosterone	42.3 11.5	0.003	0.024	0.599	0.572±0.015	42.3	44.3	44.4
Cortisone Adrenosterone	18.0 96.0	0.203	0.224	0.284	0.270±0.013	18.0	18.9	32.4
Cortisone Testosterone	18.0 182.0	0.083	0.094	0.250	0.270±0.013	18.0	16.9	23.9
Cortisone 6-β,17-α-OH-progesterone	18.0 132.0	0.007	0.016	0.294	0.270±0.013	18.0	19.6	21.0
Tetrahydrocortisone Adrenosterone	21.0 96.0	0.176	0.193	0.305	0.281±0.005	21.0	22.8	35.9
Tetrahydrocortisone Testosterone	21.0 182.0	0.073	0.087	0.306	0.281±0.005	21.0	22.8	28.3

* Optical density (510 mμ).

TABLE 4. INCREASE IN OPTICAL DENSITY DEVELOPING BETWEEN 30 AND 60 MINUTES WHICH COULD BE ATTRIBUTED TO STEROID WHICH HAD BEEN ADDED TO URINE EXTRACTS

1			2	3	4	5
Sample	No. hrs.	Micrograms	Optical density		Change of O.D. between 30 and 60 min. attributable to added steroid	
			30 min.	60 min.	Found	Theoretical
Cortisone		34	0.481 ± .003	0.479 ± .006		
Neutral Extr. 167	0.045		0.248	0.285 ± .007		
N.E. 167 + Cort.	0.045	34	0.790 ± .018	0.811 ± .005	−0.016	0.0
N.E. 189	0.093		0.220	0.228 ± .016		
N.E. 189 + Cort.	0.093	34	0.743 ± .006	0.730 ± .003	−0.021	0.0
N.E. 227	0.304		0.256 ± 0.000	0.302 ± 0.000		
N.E. 227 + Cort.	0.304	34	0.693 ± .013	0.724 ± 0.010	−0.015	0.0
Testosterone		288	0.180 ± .006	0.350 ± .002		
N.E. 167	0.045		0.248	0.286 ± .007		
N.E. 167 + Test.	0.045	288	0.403 ± .004	0.604 ± .002	+0.163	+0.170
N.E. 189	0.093		0.220	0.228 ± .016		
N.E. 189 + Test.	0.093	288	0.369 ± .004	0.537 ± .005	+0.160	+0.170

tion of alpha-ketols is the increment of optical density between thirty and sixty minutes of reaction. From the previous discussion of the straight-line characteristics of the reactions of the slowly reducing non-ketolic compounds, it follows that this increment should be the same as that during the first thirty minutes. Therefore, if the amount of this increase is subtracted from the total density at thirty minutes, the remainder should be due to the alpha-ketols. Table 3 illustrates the degree of accuracy which may be attained by such correction. It will be noted that the corrections were insignificant when relatively small quantities of non-ketolic steroids were present. When relatively large quantities of non-ketolic reducing steroids were present, the corrected estimations always varied from the predicted values by less than 10 per cent. If the uncorrected optical densities at thirty minutes had been taken as a measure of alpha-ketols alone, overestimations ranging as high as 60 per cent would have resulted.

The amount of BT reduced by most neutral extracts of urine increases gradually in a linear fashion as the time of reaction is prolonged. This suggests the presence of some slowly reacting non-ketolic substance, which may be steroid or not. In Table 4 are recorded the optical densities obtained when reduction of BT was carried out with several neutral extracts alone and after addition of cortisone or testosterone. In each instance optical densities obtained with the mixtures agreed closely with the sum

of the densities observed when extracts and steroids were treated separately. Furthermore, the changes of optical densities attributable to the added steroids were very near the theoretical changes, as will be noted in columns 4 and 5 of Table 4.

DISCUSSION

The differences in reduction of blue tetrazolium by various groups found on steroid molecules has been discussed at length by Meyer and Lindberg (12). Whether one wishes to accentuate or minimize these differences depends on the objective of the procedure. We were prompted to undertake these investigations because of the inability to account for the total reduction produced with certain steroid extracts by their apparent alpha-ketol content.

Under the circumstances of the investigation, it was found that the optimum conditions were different for each group of BT-reacting steroids studied. In making a decision as to which of these concentrations to use in measuring the ketols, the investigator would be influenced by the extraneous factor he is most interested in minimizing, such as the alcohol blank or the contribution from Δ^4,3-ketosteroids. Conditions were thought to be most satisfactory for measurement of ketols when the concentration of the T.M.A.H. reagent was 1.0 per cent. At the thirty-minute reaction time the ketols had reacted maximally, whereas the Δ^4,3-ketosteroids had produced relatively small amounts of color.

Under these conditions our results agree with those of Zaffaroni (13), who found the same reactivity with all the ketols studied. This avoids the complications arising from the varying optical densities at times reported for both the blue tetrazolium and the Porter-Silber reactions (7, 11, 14). The modification described is more sensitive than that of Mader and Buck (11). The sensitivity is not quite as great as with the choline method described by Chen and associates(9). The latter procedure, however, involves higher blanks and requires two hours for maximum color development. This would probably not allow correction for Δ^4,3-keto compounds, as suggested elsewhere in this paper.

The slight, but definite and slow reactivity of Δ^4,3-keto compounds is in agreement with the findings of Chen *et al.* (9) and Meyer *et al.* (12) but contrary to the findings of Mader and Buck (11) and Zaffaroni (13). It might be stated here parenthetically that high and inconstant blanks and variations of reaction time may overshadow the color contributions of the Δ^4,3-ketosteroids under some conditions.

The degree of reduction depended to some extent on the presence of other functions on the molecule. Although we did not have enough compounds for complete analysis, our observations were of considerable interest.

The reduction caused by the Δ^4,3-keto group of testosterone was enhanced to a considerable extent by the presence of a 17-keto group. With a third keto group placed at carbon 11 in the case of adrenosterone, we obtained the greatest amount of reduction of the non-ketolic compounds studied.

When the 17-keto or 11-keto groupings were present on the molecule with a saturated A ring, no reduction was noted, as in the cases of androstane-17-one, androsterone or 11-keto-etiocholanolone.

It is of further interest that the introduction of a ketone or beta-hydroxyl group at carbon number 6 was found to diminish the reducing power of compounds.

When mixtures of steroids containing alpha-ketols and small or moderate amounts of Δ^4,3-ketosteroids are analyzed, the contribution of color by the latter may be minimized by stopping the reaction at thirty minutes. In the presence of disproportionately large amounts of non-ketolic steroids with a conjugated 3-keto group, the measurement of the ketols can become a problem. Observations of the behavior of these interfering steroids in the BT reaction have shown that whenever the concentration of the T.M.A.H. reagent was 1.0 per cent or less, the Δ^4,3-ketosteroids reacted continuously and uniformly during the first sixty minutes (Fig. 1). Since the ketols reach a maximum color by thirty minutes, measurement of the difference in color development between thirty and sixty minutes should indicate the amount of reduction due to non-alpha-ketolic steroids present in the mixture. This would be true if the reactivity of one compound is not changed by the presence of another compound having one or more of the configurations discussed. The mixtures of compounds cited previously were designed to test this hypothesis. Since the reduction produced by each mixture did not differ significantly from the sum of the reductions produced by the individual compounds, the proposed calculations should be valid.

Results with mixtures containing neutral extracts of urine and steroids were the same as with mixtures of known steroids. It should be emphasized, however, that this does not preclude the possibility of neutral extracts containing substances which have reducing properties similar to the alpha-ketols.

SUMMARY

1. A study is presented of the influence of different concentrations of alkali on the speed and extent of reduction of blue tetrazolium by various C_{21} and C_{19} steroids over a period of two hours at 25° C.

2. When tetramethylammonium hydroxide (T.M.A.H.) was used as base, the speed of reduction caused by alpha-ketols increased until the concentration of the base in the reaction mixture was 0.022 N (reagent

concentration 1 per cent). At very high or low concentrations, maximum reduction could not be obtained.

3. Some differences were noted between saturated alpha-ketols and those unsaturated in ring A. When the reaction was stopped at thirty or sixty minutes and the concentration of T.M.A.H. in the final reaction mixture was 0.022 N (reagent concentration 1 per cent) the variation among all the ketols was minimal and probably not significant.

4. The rate of reduction caused by non-ketolic steroids with a conjugated 3-keto group was also influenced by the concentration of alkali, but was always much slower than with the alpha-ketols. At 1 per cent reagent concentration (reaction mixture, 0.022 N) the rate of reaction was a straight-line function for the first sixty minutes.

5. Addition of an 11 and and/or 17-keto group enhanced the reaction of the Δ^4,3-ketosteroids. Addition of a 6-keto or 6-beta-hydroxy group decreased the reaction of these compounds.

6. An oxygen function at carbon 3, 11, 12, 17 or 20 was unreactive or essentially so, unless an alpha-ketol or the Δ^4,3-keto group was also present.

7. The 20,21-"diols" and 17,20,21-"triols" were also unreactive.

8. Mixtures of ketolic steroids with slowly reacting non-ketolic steroids produced a reduction equivalent in amount to the sum of the reductions produced by the compounds separately.

Acknowledgments

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GONADAL DYSGENESIS WITH MENSTRUATION: A REPORT OF 2 CASES

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THE syndrome of gonadal dysgenesis has lately been recognised as a very common cause of primary amenorrhea. Aided by the "nuclear sexing" method, described by Barr and his associates (1–4) and so brilliantly applied by Polani *et al.* (5), clinicians have been able to diagnose the condition in many cases in which the hitherto acceptable criteria of classic Turner's syndrome have been lacking. Thus, the typical vestigial gonadal ridges have been reported in patients who are not short (6–11); in many who lack the associated congenital stigmata (8, 11); in some with apparently good breast development (6, 9, 11–13); in those with a high, normal or low urinary gonadotropin (FSH) excretion (11, 13, 14); and, recently, in the presence of androgenic manifestations (8, 15, 16), or even of pseudohermaphroditic external genitalia (17).

The histologic appearance of the gonads has not been uniform. The most primitive vestiges have been composed of stromal tissue without primordial follicles; in some cases, there has been evidence of more mature development with tunica albuginea, primordial follicles and follicular cysts (7, 11, 18). In others, again, some maturation has occurred in a male direction (8, 15, 16).

REPORTS IN THE LITERATURE OF MENSTRUAL BLEEDING IN GONADAL DYSGENESIS

A review of many series of cases shows that it is rare for the condition to be diagnosed, except when there has been primary amenorrhea. Isolated patients have been reported who had very scanty menstrual bleeding. Albright, Smith and Fraser (19) refer to a 21-year-old girl who had a slight bloody vaginal discharge on a few occasions. A patient reported by Varney, Kenyon and Koch (12) had menstruated three or four times between the ages of 14½ and 16 years; she also manifested some breast development. Lisser *et al.* (9) mention 2 patients who each had one episode of scant uterine bleeding.

In our series of over 30 cases of gonadal dysgenesis, 2 patients claimed to have irregular menstrual bleeding over periods of five and six years respectively. In both the diagnosis was confirmed at laparotomy.

CASE REPORTS

Case 1

J.T. (Fig. 1), aged 21 years. This attractive and intelligent trained hospital nurse presented because of secondary amenorrhea. Her periods had appeared at the age of 15 years and menstruation ensued at intervals of one to three months for the next five years. Blood loss, which extended over three days as a rule, was moderate in amount and was unassociated with abdominal cramps or discomfort of any sort. At the age of 20 years these periods ceased.

The patient was 58 inches tall, with a pleasant facial appearance and manner. The breasts were hypoplastic with inverted nipples surrounded by pale, pink areolae. The chest was broad, but there were none of the characteristic congenital stigmata of Turner's syndrome. The external genitalia were normal. Vaginal examination disclosed a markedly atrophic mucosa.

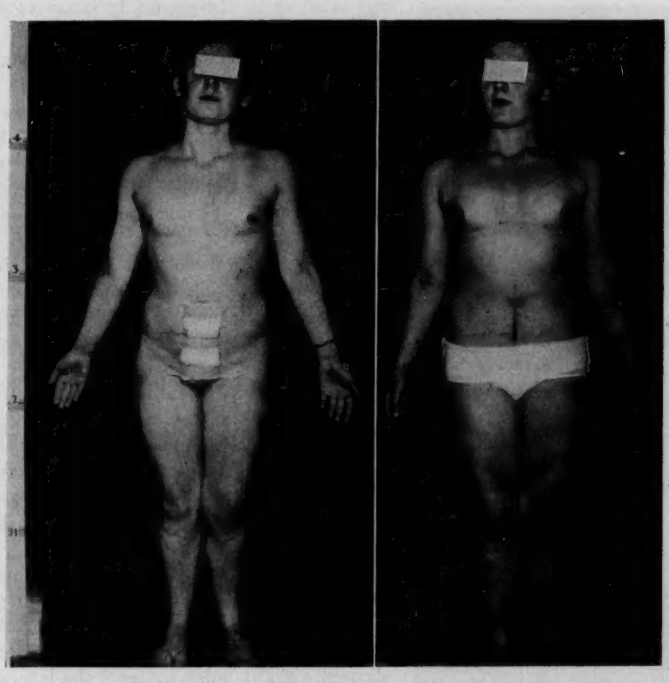

FIG. 1 FIG. 2

FIG. 1. *Case 1*, J.T., before therapy.

FIG. 2. *Case 1*, J.T., after six months of estrogen therapy. Note enlarged breasts and dark, prominent areolae.

The titer of urinary gonadotropin (FSH) excretion was positive at 96 mouse uterine units per twenty-four hours. In view of this, and the history of secondary amenorrhea, a form of secondary ovarian failure was diagnosed. There was no history of mumps and the patient denied the occurrence of hot flushes.

The nuclear pattern of the skin was of the male type. Laparotomy disclosed a minute uterus and the vestigial streaks typical of gonadal dysgenesis lying on the posterior aspects of the broad ligaments. Histologic examination of these streaks showed stroma resembling that found in adult ovaries, but it was scanty in amount. Follicles and undoubted interstitial cells were not seen.

Estrogen therapy was instituted (1 mg. of stilbestrol daily). Within six months there was an impressive increase in breast size, the areolae had enlarged and darkened (Fig. 2), cyclical withdrawal bleeding had been established and general feminization of the contours had occurred.

Case 2

A.B. (Fig. 3), aged 22 years. This patient's history was remarkably similar to that of J.T. The menarche had occurred at the age of 15 years. Irregular periods had followed for the next six years. These lasted one to three days, were generally scant in amount and came on at one-month to three-month intervals; they were completely painless. There had been several amenorrheic episodes of up to four months' duration. The patient had been amenorrheic for six months prior to admission to the hospital.

FIG. 3. *Case 2,* A.B. Note eunuchoid proportions and complete lack of breast development.

In early childhood she had suffered from mumps, but no details of this illness were available. She could not remember having had abdominal pain.

On examination she was 70 inches tall, with eunuchoid proportions (arm span 73 inches; symphysis pubis to ground 37 inches; top of head to symphysis pubis 33 inches). Axillary and pubic hair was present in moderate amount.

The most striking feature was the complete lack of development of the breasts. Two very small nipples with pale areolae were present, but no underlying mammary tissue could be palpated.

Examination of the blood smear showed female type leukocytes. The titer of urinary FSH was negative at 6 mouse uterine units.

Laparotomy revealed dysgenetic gonads lying in the site normally occupied by the primitive ridges. However, they differed from the usual "streaks" seen in this condition, in that they showed a slight bulge towards the lateral ends, a shape somewhat reminiscent of an Indian club.

Biopsy specimens were taken from the bulging ends of the gonads. Histologic examination showed that both ovaries contained numerous primordial

follicles and occasional follicular cysts. In addition, the left ovary contained at least two developing follicles.

DISCUSSION

The unexpected history of menstruation (oligomenorrhea) in patients with gonadal dysgenesis is most difficult to explain. Perhaps the simplest course is to doubt the veracity of the patients. However, both impressed us as being unusually intelligent and reliable witnesses. Supportive evidence for the occurrence of spontaneous menstrual bleeding was found in the case of A.B. who, on the first two days following laparotomy and bilateral ovarian biopsy, bled from the vagina.

It is clear that in both instances the gonads were abnormal, in the direction of immaturity. The gonads of J.T. resembled the typical streaks of tissue found in gonadal dysgenesis; in addition, the nuclear pattern of her skin was male and the FSH excretion was high. Doubts may be entertained in the case of A.B., since the leukocyte pattern was female, and the histologic examination of the gonads revealed considerable development along female lines. However, both the position and the shape of her gonads favor primary dysgenesis rather than a later secondary or postnatal failure. It is interesting that Grumbach, Van Wyk and Wilkins (17) refer to a patient with pseudo-hermaphroditic external genitalia who had identically-shaped "Indian club" gonadal vestiges.

If one accepts these gonads as primitive vestiges (and in the case of J.T. there can be no alternative), one must somehow explain the menstrual bleeding. In the absence of estrogen, menstruation should not occur. The postoperative withdrawal bleeding in A.B. suggests that some estrogen was being produced by the ovaries. Both patients denied having taken any form of hormonal preparation during the years of menstruation. Further evidence against the presence of any significant amount of circulating estrogen is provided by the rapid breast development in J. T. in response to small doses of estrogen. Normally, breast tissue is more sensitive to estrogen than is the endometrium, as indicated by breast development preceding menstruation at puberty. Presumably in these 2 patients there was sufficient circulating estrogen (of gonadal or possibly of adrenal origin) to produce endometrial bleeding, but insufficient for mammary and uterine growth.

CONCLUSION

We referred earlier to the changing ideas regarding the syndrome previously called "ovarian agenesis." The concept has been widened to include cases with a striking variety of clinical features. To our knowledge, our 2 patients are the first to be described with menstrual bleeding (oligo-

menorrhea) over prolonged periods of time. Undoubtedly gonadal dysgenesis is assuming a position of great importance as a cause of primary amenorrhea. It would appear that one must consider it, too, in some patients with secondary amenorrhea.

SUMMARY

Two patients are described in whom it is averred that menstruation took place over periods of five and six years respectively. In both cases, at laparotomy, gonadal dysgenesis was shown to exist. Some possible mechanisms for the menstrual bleeding are discussed but we are unable to offer any satisfactory explanation.

It is interesting that both patients lacked the typical anomalies of Turner's syndrome, and one was of normal height.

Acknowledgments

We are pleased to acknowledge our gratitude to Professor James T. Louw and Professor F. Forman, under whose care these patients were admitted; to Dr. C. J. Uys and Dr. M. Sachs for the histologic reports; to Dr. R. Sharpe for referring *Case 1;* to Dr. M. J. Bailey for referring *Case 2;* to Prof. D. Stammers for the leukocyte sexing; to Prof. H. Zwarenstein and Mr. D. G. Duncan for the gonadotropin estimations; and to Mr. B. Todt for the photographs.

Addendum

Since we submitted this paper we have seen a patient (J.Thw.) similar in all respects to Patient A.B. (Case 2), except that she complained of primary amenorrhea. She also had tall eunuchoid proportions, undeveloped breasts, female nuclear pattern and low FSH excretion. Laparotomy disclosed almost identical "club-shaped" gonads, which consisted histologically of stroma containing a few primordial follicles and follicular cysts.

In retrospect we believe these 2 patients (A.B. and J.Thw.) to be suffering from intrauterine pituitary gonadotropic failure causing incomplete development and slight ectopia of the ovaries. This would place them in a category (hypogonadotropic hypogonadism) distinct from that of J.T. (Case 1).

It is a moot point whether the term "gonadal dysgenesis" should be stretched to include such examples of partial ovarian failure with low FSH excretion. Since the abnormality appears to be developmental it is probably logical to do so.

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Letters to the Editor

EFFECT OF ESTROGEN THERAPY ON CERULOPLASMIN CONCENTRATION IN A MAN WITH WILSON'S DISEASE

To the Editor:

It has been demonstrated in this laboratory by Russ and Raymunt (1) that a two- to three-fold increase in the concentration of total serum copper and ceruloplasmin could be obtained following administration of estrogen as ethinyl estradiol (Estinyl) in doses ranging from 0.25 mg. to 1.0 mg. daily. The concentrations thus obtained were greater than those observed in any previously reported conditions, even in the third trimester of pregnancy and in leukemia—states in which ceruloplasmin values are known to be very high.

The authors thought it of interest to attempt to augment through the use of estrogens the ceruloplasmin level in hepatolenticular degeneration or Wilson's disease, a condition in which there is a marked deficiency of this oxidase.

The method used for the determination of total serum copper concentration was the diethyldithiocarbamate procedure of Gubler and co-workers (2). The "indirect" reacting fraction presumably corresponds to ceruloplasmin, and normally represents 90–100 per cent of the total copper (3).

The subject chosen for the experiment was a 42-year-old white male patient (Mr. F.S.) who was known to have had Wilson's disease for at least five years. He was one of 12 siblings. Three sisters and 1 brother had Wilson's disease but his mother and father, who were first cousins, were not afflicted, and so far as could be determined, their families were also free of the disease. The patient improved, both neurologically and mentally, after several courses of BAL (British antilewisite) therapy, but no change was noted in the abnormal results of the liver function tests. BAL was discontinued nine weeks prior to the introduction of estrogen therapy, at which time the patient's condition appeared satisfactory.

He was then given 1 mg. of Estinyl daily. After one week he began to complain of painful, tender, enlarged breasts. During the next several weeks his neurologic and mental condition became progressively worse. Tremors increased, and he became restless, disinterested, forgetful and

TABLE 1. EFFECT OF ESTROGEN THERAPY ON SERUM LEVELS OF TOTAL COPPER AND OF CERULOPLASMIN IN A MAN WITH WILSON'S DISEASE

Copper (γ/100 ml.)		Treatment and duration
Total	Ceruloplasmin ("indirect") fraction	
72	58	None
77	77	None
70	52	2 wks.
67	33	4 wks.
64	33	6 wks.
48	27	9 wks.
		Estinyl, 1.0 mg. daily

depressed. He began to complain of loss of libido. The breasts, although they remained enlarged, apparently were no longer painful or tender. Medication was discontinued after a period of nine weeks.

In Table 1 are listed the levels of total serum copper and ceruloplasmin ("indirect" fraction) before and during the administration of Estinyl.

The results are in contrast to those previously reported (1) in estrogen-treated subjects with conditions other than hepatolenticular degeneration. In this typical case of Wilson's disease, there was no augmentation of serum ceruloplasmin concentration during administration of estrogen.

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EFFECT OF ANDROGENS ON α_2-GLOBULINS IN WOMEN

To the Editor:

Recently Sachs, Danielson and Weston (1) and v. Studnitz and Berezin (2) reported an increase in the levels of the α_2-globulins in human serum following the administration of anabolic steroids and androgens, respectively. The former authors used a new synthetic steroid, 17-ethyl-19-nortestosterone, whereas the latter used methylandrostenediol, methyltestosterone and testosterone propionate. Since it is now possible to determine at least 2 of the probably 4 or 5 components of the α_2-globulins, namely ceruloplasmin and haptoglobin, it was decided to investigate whether the increase in the level of α_2-globulins following the administration of androgen is related to either or both of these components.

Ceruloplasmin, the copper protein, normally occurs in a concentration of about 30 mg. per 100 ml. of serum. Haptoglobin (Jayle and Boussier (3) is an α_2 mucoprotein which forms a strong complex with hemoglobin *in vivo* and *in vitro*. The reaction is stoichiometric (1 gram of haptoglobin binds 0.83 gram of hemoglobin). The haptoglobin content of the serum is normally 30 mg. to 180 mg. per ml. and thus corresponds to 10–30 per cent of the α_2-globulins.

MATERIAL AND METHODS

The material consisted of sera from 10 females in whom, after the administration of androgen, there was an increase in the serum level of α_2-globulin. The ceruloplasmin level was calculated from the copper content (1 mol binds 8 atoms of copper) (4). Haptoglobin was determined by the method of Jayle (3).

The results are listed in Table 1. In 9 of the 10 cases, administration of androgen was associated with an increase in the serum concentration of α_2-globulin complex. In 8 of these 9, an increase was also noted in the haptoglobin content, whereas the ceruloplasmin content showed only slight variation in either direction. Only in 1 case (No. 10) was there no increase in the haptoglobin level despite the rise in the α_2-complex; in this instance, there was also no change in the ceruloplasmin values.

In Cases 4, 8 and 9, determinations were made after withdrawal of the androgen therapy. A decrease in the haptoglobin content was noted in all 3 cases, parallel with the decrease in the α_2-complex. In Case No. 3 hormone treatment was followed immediately by a second course, and then a further increase was noted in the levels of α_2-complex and haptoglobin.

TABLE 1

Case	Therapy*	Period† (days)	Serum level (mg./100 ml.)			
			α_2 Globulins	Haptoglobin	α_2-Glob. minus Hapto.	Ceruloplasmin
1	1)		540	160	380	22
	2) MA	18	620	280	340	55
2	1)		450	160	290	40
	2) MA	28	640	390	250	47
3	1)		480	70	410	32
	2) MA	30	520	190	330	34
	2) MA	10	570	200	370	34
4	1)		510	160	350	30
	2) MA	27	670	260	410	19
	3) After	32	570	150	420	25
5	1)		500	220	280	47
	2) MT	36	680	380	300	47
6	1)		470	140	330	42
	2) MA		530	210	320	31
7	2) TP	10	500	180	320	22
	3) After	16	470	130	340	47
	3) After	10	470	160	310	45
8	1)		480	70	410	30
	2) MT	20	620	240	380	65
	3) After	30	450	70	380	57
9	1)		440	160	280	44
	2) MT	20	550	220	330	41
	3) After	30	440	100	340	47
10	1)		460	90	370	35
	2) MA	20	530	80	450	35

* 1) = Values before androgen therapy.
2) = Values during androgen therapy.
3) = Values after androgen therapy.
MA = Methylandrostenediol.
MT = Methyltestosterone.
TP = Testosterone propionate.
† Number of days of androgen therapy or of omission, respectively.

COMMENTS

From Table 1 it may be seen that, despite the slight variation due to the error of the methods, the increase in the level of α_2-globulins could be ascribed entirely to the increase in the level of haptoglobin. Also the decrease in the α_2-proteins on withdrawal of androgen therapy was due to the decrease in the concentration of haptoglobin. To our knowledge, haptoglobin levels have never been determined following the administration of androgen. Our results agree, however, with the observations of Greenspan (5) and Kelley (6) for example, who found a positive correlation between adrenocortical function and the mucoprotein content of the serum (it is not yet known to what extent haptoglobin is included in the mucoproteins as determined by the method of Winzler).

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CYCLIC CHANGES IN FEMALE SALIVA

To the Editor:

The process of arborization of the mucus from the human uterine cervix has been demonstrated by Papanicolaou (1), Rydberg (2), Zondek and Rozin (3) and others. The fern-leaf patterns of the cervical mucus appear during estrogenic stimulation and terminate with ovulation and luteal activity.

Recently Henderson (4) made an investigation into the cyclic changes in female nasal mucus, and observed a fern-leaf pattern in the smears during the estrogenic phase and disintegration of the arborization during the secretory phase of menstrual cycle.

We have made a study of the crystallization of saliva in 10 female volunteers. These women, aged 18–26 years, had regular menstrual cycles

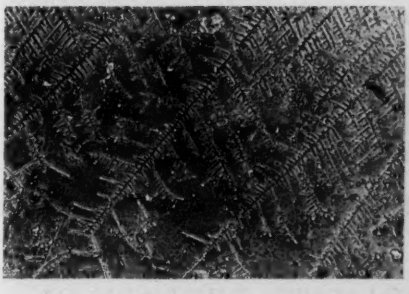

Fig. 1

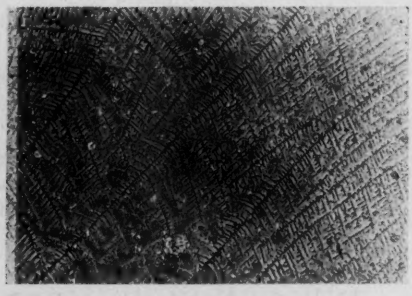

Fig. 2

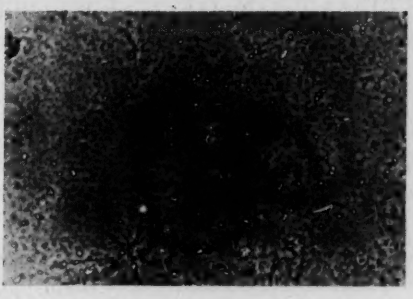

Fig. 3

Smears of Saliva from a Woman with Regular Menstrual Cycle.

Fig. 1. Fourth day of cycle (during menstrual bleeding); initial arborization.
Fig. 2. Fourteenth day of cycle: marked increase of arborization.
Fig. 3. Twentieth day of cycle: no arborization
(All photomicrographs of the same magnification: ×200.)

with ovulation occurring between the fourteenth and sixteenth days, as determined by the basal temperature readings and urinary pregnanediol determinations. Saliva was obtained daily and smeared on a clean slide.

Fern-leaf patterns were observed in the saliva during the estrogenic phase of the menstrual cycle (Figs. 1 and 2). After ovulation, in the secretory phase, these patterns were no longer evident (Fig. 3).

The results of this study suggest that, in the human female, arborization occurs not only in the uterine cervical mucus and in the nasal mucus, but in the saliva.

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INFLUENCE OF MEPROBAMATE THERAPY ON THE ESTIMATION OF 17-KETOSTEROIDS AND 17-KETOGENIC STEROIDS

TO THE EDITOR:

We have made the following observations:

1. Meprobamate (2-methyl-2*n*-propyl-1,3-propanediol dicarbamate), one of the new tranquilizing drugs, produces a characteristic reaction with the Zimmermann reagent, with an absorption maximum at 395 mμ. Using quantities from 250 to 1000 γ, the reaction follows Beer's law, and may thus be used in the quantitative estimation of meprobamate.

2. When ingested, meprobamate is excreted in the urine, and is found in the final urinary extracts for colorimetry in the assay for 17-ketosteroids according to the method of Vestergaard (1), and in the assay for 17-ketogenic steroids according to a modification of the method of Norymberski described by Diczfalusy *et al.* (2).

The optical density of meprobamate in the Zimmermann reaction—between 350 mμ and 450 mμ—makes the use of Gibson and Evan's (3)

correction formula (readings at 420 mμ and 520 mμ) valueless on urine from meprobamate-treated patients. With application of the correction formula of Allen (4) and readings at 470, 520 and 570 mμ, however, meprobamate in the usual therapeutic doses has no significant influence on the values obtained for urinary 17-ketosteroids and 17-ketogenic steroids.

A detailed report of our observations will be published in *Acta endocrinologica.*

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RECIPIENTS OF THE 1957 AWARDS

THE MEDAL OF THE ENDOCRINE SOCIETY

Dr. Joseph Charles Aub, past president of the Endocrine Society, has been a continuous contributor to endocrinology throughout his long and active life. Born in 1890 in Cincinnati, Ohio, he graduated with an A.B. degree from Harvard College in 1911 and an M.D. degree from the Harvard Medical School in 1914. Following a medical internship at the Massachusetts General Hospital in Boston, with Dr. Eugene DuBois he worked out the surface area formula for the calculation of metabolic rate, and with Dr. Dennis the disturbed glucose metabolism of the patient with hyperthyroidism. With a research group in France during World War I he studied the lowered metabolic rate of the state of shock. After the war, as Assistant Professor of Physiology, he made his classic studies in lead poisoning. He and his co-workers described the passage of lead with calcium into and out of the bones. These studies laid the foundation not only for the modern therapy of lead poisoning but also for the clinical studies of calcium and phosphorus metabolism. With Dr. DuBois and their colleagues he described the first case of hyperparathyroidism in this country at a time when the discovery of Mandl was still unknown. He also was the first to recognize the effect of thyroid and of pituitary hormone on calcium metabolism.

In 1928 he was appointed Director of the Huntington Laboratories of Cancer Research at Harvard University and was one of the first to approach the problem of cancer from the point of view of endocrinology. Hormone studies were made of the growth and development of children and of patients suffering from various cancers. With Dr. Ira Nathanson he was in the forefront of treating breast cancer with hormones. His earlier studies in calcium led to those of radium and other heavy metals.

In 1942, when the Huntington Laboratories moved to the Massachusetts General Hospital, he was promoted to Professor of Research Medicine. The endocrine studies of cancer continued under his direction. His publications, now numbering 176, cover a wide range of subjects involving growth and metabolism. Retired from his chair of medicine in 1956, he has returned to the laboratory to study the cell membrane.

AWARDED
THE MEDAL OF THE ENDOCRINE SOCIETY

DR. JOSEPH C. AUB

In order to judge the fullness of his accomplishment one has to look at the long list of investigators who at one time or another have studied under him and have been inspired by him. Many are members of The Endocrine Society and many are professors of medicine in medical schools throughout United States and Canada. His greatest interest has been these his young colleagues and to them he has brought the great warmth of his personality.

DR. NICHOLAS S. HALMI

THE CIBA AWARD

The Ciba Award for 1957 goes to *Dr. Nicholas S. Halmi*, who is a worthy addition to the group of young endocrinologists so honored by The Endocrine Society.

Dr. Halmi's capacity for acute perception, objective reasoning, and fluent expression are native endowments derived from an equally gifted father. The manner in which he has employed those talents to carve for himself a brilliant career in science must be credited to himself alone.

Dr. Halmi was born in Budapest, June 6, 1922, where he received his primary and secondary education. He attended medical school at the Universities of Kolozsvár (Chuj), Transylvania, and Budapest, where he graduated "sub laurea Almae Matris" in 1947, despite the fact that during the German occupation of Hungary he was taken into custody by the Gestapo and after his release was obliged to go underground. During his intern training in Budapest, he also worked for two years as a research assistant in pathology at St. Johns Hospital and in 1947 became assistant to Professor J. Szentagothai in the Department of Anatomy, University of Pecs, where his interest in the study of hypophysio-hypothalamic relationships was born. Dr. Halmi and his father, who had been prominent in the financial and cultural affairs of pre-Communist Hungary, were under constant surveillance by the Communists during this period. In January 1949, the pressure became very acute and after it became evident that they were about to be deported to Siberia, the Halmis escaped in a commandeered plane to Munich and later that year Dr. Halmi reached the United States. In 1955 he became a naturalized citizen.

From 1949 to 1950 Dr. Halmi was an instructor in anatomy at the University of Chicago. Since 1950 he has been a member of the Department of Anatomy, State University of Iowa, where he now holds the rank of

Associate Professor and where he is recognized as a gifted and stimulating teacher, as well as a brilliant investigator.

Dr. Halmi's studies have been focussed primarily in the area of his early interest in hypophysio-hypothalamic relationships, but his inquiring mind has ever turned to new methods of approaching this complex subject, as well as other problems of endocrine physiology. Thus his interests have included various studies on the cytophysiology of the hypophysis and hypothalamus, on the effect of hypothalamic lesions in various aspects of hypophysial function, on the relation of hormonal and dietary factors to carbohydrate metabolism, and on the relation between intrinsic and hypophysial factors in the regulation of the iodide-concentrating mechanisms of the thyroid. Although his more recent work has been in other areas, Dr. Halmi continues to be regarded as an authority on the cytophysiology of the hypophysis. His contributions to this subject include the recognition of differences in morphology and function of the beta and delta cells of the rat pituitary, as well as clearly defined demonstrations of the response of the pituitary to a variety of experimental conditions. His studies on modification of hypophysial function by lesions in the hypothalamus ranged through the entire endocrine system and were particularly valuable in regard to effects on carbohydrate metabolism, and thyroid and gonadal function. Some of these findings led to important studies on the relation of dietary and hormonal factors to carbohydrate metabolism. By no means the least significant of his investigations have been those concerning the iodide-concentrating mechanisms of the thyroid. His work on iodide trapping as influenced by serum iodine levels and thyrotropic hormone represents an important advance in our understanding of thyroid physiology and has become the basis of a widely used technique in studies of thyroid function. During the last six years, he has published approximately 30 papers, each of which has represented a carefully executed study reported in a skillful and meticulous fashion. His command of our language is such that his American-born colleagues have eagerly accepted his suggestions for changes in their own manuscripts.

Dr. Halmi's contributions to the advance of experimental endocrinology have been significant and valuable, both for their content and for the stimulus they have provided to further investigations. We may be certain that his capacity for original thinking and for skillful execution of his ideas will combine to provide us with many more important studies.

DR. GIOVANNI G. COSTA

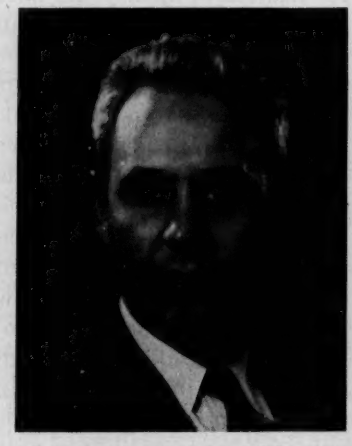
THE AYERST FELLOWSHIP

Dr. Giovanni Costa is the recipient of the Ayerst Fellowship for 1957, presented by The Endocrine Society.

Dr. Costa was born in Collagna, Italy, February 6, 1929 and received the degree of M.D., Maxima Cum Laude, from the University of Genoa School of Medicine in 1953. His M.D. thesis was entitled "Action of Cardiac Glucosides on Heart Metabolism in Vitro." During 1954 he worked under the direction of Professor Hans Selye and completed a study entitled "Conditioning of the Gluconeogenetic Properties of Cortisol by Stress." Since October 1954, he has been a Research Assistant at the Oklahoma Medical Research Foundation under the direction of Dr. C. D. Kochakian, and he has been given the Fellowship to permit him to continue his studies there on the mechanism of protein synthesis under androgen stimulation.

THE SCHERING SCHOLAR

The Schering Scholar of The Endocrine Society for 1957 is *Dr. Gerald Conrad Mueller*, Associate Professor in the Department of Oncology, University of Wisconsin Medical School.



DR. GERALD CONRAD MUELLER

Dr. Mueller plans to study the factors which regulate the biosynthesis of intracellular particulates and to learn about the interrelationship of nucleic acid and protein synthesis and factors which alter this relationship. For this purpose he hopes to spend six months at the Max-Planck Institut für Virusforschung in Tübingen, Germany, studying virus reproduction and the metabolic interaction of the virus particle with the host cell. In addition, opportunity will be taken to visit and discuss problems of endocrinology and cell physiology with leading European scientists.

THE UPJOHN SCHOLAR

DR. RACHMIEL LEVINE

The Upjohn Scholar of The Endocrine Society for 1957 is *Dr. Rachmiel Levine*, Director of the Department of Metabolic and Endocrine Research, and Chairman of the Department of Medicine, Michael Reese Hospital, Chicago; and Professorial Lecturer, Department of Physiology, University of Chicago.

Dr. Levine plans to visit the laboratory of Dr. Walter Wilbrandt in Bern, Switzerland and that of Dr. Ussing in Copenhagen, for study of cell membrane structure and the general field of permeability, with a view to applying some of the techniques to the subject of transfer of sugars across cell barriers, specifically those of skeletal muscle and of fat tissue.

THE 1958 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada. Dr. Carl R. Moore was the recipient in 1955, Dr. Frederick L. Hisaw in 1956, and Dr. Joseph C. Aub in 1957.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was se-

lected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni; 1957—Dr. Nicholas S. Halmi. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such ap-

plications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1* each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

NINTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY AND THE MEDICAL COLLEGE OF GEORGIA

Augusta, Georgia, October 21-25, 1957

The faculty will consist of 24 eminent clinicians and investigators from various parts of the country in the fields of endocrinology and metabolism. The program will cover the various endocrinopathies, with emphasis on the clinical aspects, demonstration of laboratory tests, presentations of cases, and question-and-answer panel discussions. The course is designed to cover the main aspects of diagnosis and therapy in the field of endocrinology and metabolism for the physician in general practice and for those in other specialities who wish to have a general knowledge of this rapidly growing field.

A syllabus with brief abstracts of lectures will be available to the registrants at the time of the Assembly.

For further information concerning the program and registration, write to *Dr. Robert B. Greenblatt, Department of Endocrinology, Medical College of Georgia, Augusta, Georgia.* Registration is limited to 100; the tuition fee is $100.00. Rooms will be reserved for the students and faculty at the Bon Air Hotel. Residents and fellows will be admitted for $35.00.

(For Faculty and Program, see June issue)

BOOK REGISTER

Gout. Second edition. By JOHN H. TALBOTT, M.D., D.Sc.(Hon.), Professor of Medicine, University of Buffalo School of Medicine; Physician-in-Chief, Buffalo General Hospital, Buffalo, N. Y. As stated in the preface, this book records the results of a renewed effort to describe the usual features of gout and gouty arthritis, as well as several of the unusual features. It is an extension of an earlier monograph, but has been completely re-written with a new up-to-date set of illustrations. It is hoped that the book will arouse in physicians a greater interest in the early recognition of gouty arthritis, in order to insure early and prompt treatment; 205 pages; 63 figures; 16 color plates; 1957. Grune & Stratton, Inc., New York, N. Y. Price $6.75.

Regulation and Mode of Action of Thyroid Hormones. Ciba Foundation Colloquia on Endocrinology, Volume X, edited by G. E. W. WOLSTENHOLME, M.B., B.Ch. and ELAINE C. P. MILLAR, A.H.-W.C. Papers and informal discussions by leading investigators from all over the world present ideas on the latest concepts concerning the thyroid. Among the subjects discussed are the regulation of thyroid activity, the influence of the central nervous system on the control of thyrotropin, pituitary cytology, factors influencing the thyroidal iodide pump, the "feed-back" hypothesis of the control of thyroid function, hormone synthesis in the iodine-deficient gland, the distribution and metabolism of thyroid hormones, hepatic regulation of thyroxine metabolism, cellular actions of thyroxine and similar compounds, and the influence of the thyroid upon immune responses to bacterial infection; 311 pages; 114 illustrations; 1957. Boston; Little, Brown and Company. Price $8.50.

The Chemistry and Biology of Purines. Ciba Foundation Symposium, edited by G. E. W. WOLSTENHOLME, M.B., B.Ch. and CECILIA M. O'CONNOR, B.Sc. A record of current thought on purines from the approach of the synthetic chemist and the point of view of the biochemist, possibly leading to the development of new chemotherapeutic agents whose activity depends upon their purine structure. A book of interest to biologists and to investigators in the fields of cancer and genetics; 327 pages; 124 illustrations and structural formulae; 1957. Boston; Little, Brown and Company. Price $9.00.

The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence. Second edition. By LAWSON WILKINS, M.D., Associate Professor of Pediatrics, The Johns Hopkins University, The Harriet Lane Home, The Johns Hopkins Hospital, Baltimore, Maryland. This outstanding book in its field has been extensively revised and expanded, to make it of even greater value to the pediatric clinician. There are new discussions of the adrenogenital syndrome, hermaphrodism, gonadal dysgenesis, sex chromatin, and the metabolism of carbohydrate, calcium and phosphorus. New chapters are included on the chemistry and physiology of the thyroid and adrenals, and it is emphasized that some disorders of these glands are due to abnormalities of biosynthesis dependent upon inborn defects in the respective enzymic systems. Newer diagnostic methods are discussed, and Chapter IV is an attempt to explain the chemical nomenclature concerned, and to point out the diagnostic applications and limitations of the methods of measurement. Differential diagnosis is stressed. This edition contains 526 pages; 178 figures (5 in color); 38 tables; 1957. Charles C Thomas, Publisher, Springfield, Illinois. Price $17.50.